



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup>:</b> <b>C07K 14/195, C12N 15/31, 1/21, 5/10, A01H 5/00, 5/10, C12N 15/82</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 00/20452</b> <b>(43) International Publication Date:</b> 13 April 2000 (13.04.00)
<b>(21) International Application Number:</b> PCT/US99/23181 <b>(22) International Filing Date:</b> 5 October 1999 (05.10.99) <b>(30) Priority Data:</b> 60/103,050 5 October 1998 (05.10.98) US <b>(71) Applicant:</b> EDEN BIOSCIENCE CORPORATION [US/US]; 11816 North Creek Parkway N., Bothell, WA 98011-8205 (US). <b>(72) Inventors:</b> WEI, Zhong-Min; 8230 125th Court, Kirkland, WA 98034 (US). FAN, Hao; 19712 6th Drive S.E., Bothell, WA 98012 (US). NIGGEMEYER, Jennifer, L.; 21315 2nd Avenue S.E., Bothell, WA 98021 (US). <b>(74) Agents:</b> GOLDMAN, Michael, L. et al.; Nixon Peabody LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 6 July 2000 (06.07.00)
<b>(54) Title:</b> HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE  <b>(57) Abstract</b>  The present invention is directed to isolated active fragments of a hypersensitive response elicitor protein or polypeptide which fragment does not elicit a hypersensitive response in plants. Also disclosed are isolated DNA molecules which encode such fragments. Isolated fragments of hypersensitive response elicitor proteins or polypeptides in accordance with the present invention and the isolated DNA molecules that encode them have the following activities: imparting disease resistance to plants, enhancing plant growth, and/or controlling insects on plants. This can be achieved by applying the fragments of a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the fragment can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

SEP 28 2000

## **HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE**

This application claims benefit of U.S. Provisional Patent Application  
5 Serial No. 60/103,050, filed October 5, 1998.

### **FIELD OF THE INVENTION**

The present invention relates to active fragments of a hypersensitive  
10 response elicitor which fragments do not elicit a hypersensitive response.

### **BACKGROUND OF THE INVENTION**

Interactions between bacterial pathogens and their plant hosts generally  
15 fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations  
20 increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z.,  
25 "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily  
30 observed as a tissue collapse if high concentrations ( $\geq 10^7$  cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

- 2 -

“Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf,” Phytopathology 54:474-477 (1963); Turner, et al., “The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction,” Phytopathology 64:885-890 (1974); Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., “Hypersensitivity,” pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., “Gene Cluster of *Pseudomonas syringae* pv. ‘phaseolicola’ Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants,” J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in Gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., “*hrp* Genes of Phytopathogenic Bacteria,” Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., “*hrp* Genes of Phytopathogenic Bacteria,” pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia*, *Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., “Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria,” Trends Microbiol. 1:175-180 (1993)). In *E. amylovora*, *P. syringae*, and *P. solanacearum*, *hrp* genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. “*Pseudomonas Syringae* pv. *Syringae* HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants,” Cell 73:1255-1266 (1993), Wei, Z.-H.,



- 3 -

et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al.

"PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al., "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among Gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "Erwinia chrysanthemi Harpin<sub>Ech</sub>: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA<sup>-</sup> Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1996)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

- 4 -

The present invention seeks to identify fragments of hypersensitive response elicitor proteins or polypeptides, which fragments do not elicit a hypersensitive response but are active when utilized in conjunction with plants.

5

## SUMMARY OF THE INVENTION

The present invention is directed to isolated fragments of an *Erwinia* hypersensitive response elicitor protein or polypeptide which fragments do not elicit a hypersensitive response in plants but are otherwise active when utilized in conjunction with plants. Also disclosed are isolated DNA molecules which encode such fragments.

The fragments of hypersensitive response elicitors according to the present invention have the following activity when utilized in conjunction with plants: imparting disease resistance to plants, enhancing plant growth and/or controlling insects. This involves applying the fragments in a non-infectious form to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

As an alternative to applying the fragments to plants or plant seeds in order to impart disease resistance, to enhance plant growth, and/or to control insects on plants, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA-molecule encoding a fragment of a hypersensitive response elicitor protein or polypeptide in accordance with the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects in the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the DNA molecule encoding such a fragment can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, and/or to control insects on plants or plants grown from the plant seeds.

30

- 5 -

### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows truncated proteins of the hypersensitive response elicitor protein or polypeptide.

5           Figure 2 shows a list of synthesized oligonucleotide primers for construction of truncated harpin proteins. N represents the N-terminus (5' region), and C represents the C-terminus (3' region). The primers correspond to the indicated sequence identification numbers for the present application: N1 (SEQ. ID. No. 1), N76 (SEQ. ID. No. 2), N99 (SEQ. ID. No. 3), N105 (SEQ. ID. No. 4), N110 (SEQ.  
10 ID. No. 5), N137 (SEQ. ID. No. 6), N150 (SEQ. ID. No. 7), N169 (SEQ. ID. No. 8), N210 (SEQ. ID. No. 9), N267 (SEQ. ID. No. 10), N343 (SEQ. ID. No. 11), C75 (SEQ. ID. No. 12), C104 (SEQ. ID. No. 13), C168 (SEQ. ID. No. 14), C180 (SEQ. ID. No. 15), C204 (SEQ. ID. No. 16), C209 (SEQ. ID. No. 17), C266 (SEQ. ID. No. 18), C342 (SEQ. ID. No. 19), and C403 (SEQ. ID. No. 20).

15

### **DETAILED DESCRIPTION OF THE INVENTION**

The present invention is directed to isolated fragments of a hypersensitive response elicitor protein or polypeptide where the fragments do not  
20   elicit a hypersensitive response but have other activity in plants. Also disclosed are DNA molecules encoding such fragments as well as expression systems, host cells, and plants containing such molecules. Uses of the fragments themselves and the DNA molecules encoding them are disclosed.

The fragments of hypersensitive response elicitor polypeptides or  
25   proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein  
30   elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

- 6 -

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

5 The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 21 as follows:

10	Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser	1 5 10 15
	Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser	20 25 30
	Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr	35 40 45
15	Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu	50 55 60
	Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser	65 70 75 80
20	Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys	85 90 95
	Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp	100 105 110
	Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln	115 120 125
25	Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met	130 135 140
	Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly	145 150 155 160
30	Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly	165 170 175
	Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu	180 185 190
	Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala	195 200 205
35	Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val	210 215 220
	Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp	225 230 235 240

[illegible]

	CGATTTTACC	CGGGTGAACG	TGCTATGACC	GACAGCATCA	CGGTATTCTGA	CACCGTTACG	60
	GCGTTTATGG	CCGCGATGAA	CCGGCATCAG	GCGGCGCGCT	GGTCGCCGCA	ATCCGGCGTC	120
	GATCTGGTAT	TTCAGTTTGG	GGACACCGGG	CGTGAACTCA	TGATGCAGAT	TCAGCCGGGG	180
25	CAGCAATATC	CCGGCATGTT	GCGCACGCTG	CTCGCTCGTC	GTTATCAGCA	GGCGGCAGAG	240
	TGCGATGGCT	GCCATCTGTG	CCTGAACGGC	AGCGATGTAT	TGATCCTCTG	GTGGCCGCTG	300
	CCGTCGGATC	CCGGCAGTTA	TCCGCAGGTG	ATCGAACGTT	TGTTTGAAC	GGCGGGAATG	360
	ACGTTGCCGT	CGCTATCCAT	AGCACCGACG	GCGCGTCCGC	AGACAGGGAA	CGGACGCGCC	420
	CGATCATTA	GATAAAGGCG	GCTTTTTTTT	TTGCAAAACG	GTAACGGTGA	GGAACCGTTT	480
30	CACCGTCGGC	GTCACTCAGT	AACAAGTATC	CATCATGATG	CCTACATCGG	GATCGGCGTG	540
	GGCATCCGTT	GCAGATACTT	TTGCGAACAC	CTGACATGAA	TGAGGAAACG	AAATTATGCA	600
	AATTACGATC	AAAGCGCACA	TCGGCGGTGA	TTTGGGCGTC	TCCGGTCTGG	GGCTGGGTGC	660
	TCAGGGACTG	AAAGGACTGA	ATTCCGCGGC	TTCATCGCTG	GGTTCAGCG	TGGATAAACT	720
	GAGCAGCACC	ATCGATAAGT	TGACCTCCGC	GCTGACTTCG	ATGATGTTTG	GCGGCGCGCT	780
35	GGCGCAGGGG	CTGGGCGCCA	GCTCGAAGGG	GCTGGGGATG	AGCAATCAAC	TGGGCCAGTC	840

- 8 -

	TTTCGGCAAT	GGCGCGCAGG	GTGCGAGCAA	CCTGCTATCC	GTACCGAAAT	CCGGCGGCGA	900
	TGCGTTGTCA	AAAATGTTTG	ATAAAGCGCT	GGACGATCTG	CTGGGTCATG	ACACCGTGAC	960
	CAAGCTGACT	AACCAGAGCA	ACCAACTGGC	TAATTCAATG	CTGAACGCCA	GCCAGATGAC	1020
	CCAGGGTAAT	ATGAATGCGT	TCGGCAGCGG	TGTGAACAAC	GCACTGTCGT	CCATTCTCGG	1080
5	CAACGGTCTC	GGCCAGTCGA	TGAGTGGCTT	CTCTCAGCCT	TCTCTGGGGG	CAGGCGGCTT	1140
	GCAGGGCCTG	AGCGGCGCGG	GTGCATTCAA	CCAGTTGGGT	AATGCCATCG	GCATGGGCGT	1200
	GGGGCAGAAT	GCTGCGCTGA	GTGCGTTGAG	TAACGTCAGC	ACCCACGTAG	ACGGTAACAA	1260
	CCGCCACTTT	GTAGATAAAG	AAGATCGCGG	CATGGCGAAA	GAGATCGGCC	AGTTTATGGA	1320
	TCAGTATCCG	GAAATATTCG	GTAAACCGGA	ATACCAGAAA	GATGGCTGGA	GTTCCGCCGAA	1380
10	GACGGACGAC	AAATCCTGGG	CTAAAGCGCT	GAGTAAACCG	GATGATGACG	GTATGACCGG	1440
	CGCCAGCATG	GACAAATTCC	GTCAGGCGAT	GGGTATGATC	AAAAGCGCGG	TGGCGGGTGA	1500
	TACCGGCAAT	ACCAACCTGA	ACCTGCGTGG	CGCGGGCGGT	GCATCGCTGG	GTATCGATGC	1560
	GGCTGTCTGC	GGCGATAAAA	TAGCCAACAT	GTCGCTGGGT	AAGCTGGCCA	ACGCTGATA	1620
	ATCTGTGCTG	GCCTGATAAA	GCGGAAACGA	AAAAAGAGAC	GGGGAAGCCT	GTCTCTTTTC	1680
15	TTATTATGCG	GTTTATGCGG	TTACCTGGAC	CGGTTAATCA	TCGTCATCGA	TCTGGTACAA	1740
	ACGCACATTT	TCCC GTTCAT	TCGCGTCGTT	ACGCGCCACA	ATCGCGATGG	CATCTTCCTC	1800
	GTCGCTCAGA	TTGCGCGGCT	GATGGGGAAC	GCCGGGTGGA	ATATAGAGAA	ACTCGCCGGC	1860
	CAGATGGAGA	CACGTCTGCG	ATAAATCTGT	GCCGTAACGT	GTTTCTATCC	GCCCCTTTAG	1920
	CAGATAGATT	GCGGTTTTCGT	AATCAACATG	GTAATGCGGT	TCCGCCTGTG	CGCCGGCCGG	1980
20	GATCACCACA	ATATTCATAG	AAAGCTGTCT	TGCACCTACC	GTATCGCGGG	AGATACCGAC	2040
	AAAATAGGGC	AGTTTTTTGCG	TGGTATCCGT	GGGGTGTTC	GGCCTGACAA	TCTTGAGTTG	2100
	GTTTCGTATC	ATCTTTCTCC	ATCTGGGCGA	CCTGATCGGT	T		2141

25                   The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 23 as follows:

30 Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
1 5 10 15  
Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
20 25 30

- 9 -

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
 35 40 45  
 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 50 55 60  
 5 Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80  
 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95  
 10 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110  
 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125  
 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140  
 15 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160  
 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175  
 20 Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
 180 185 190  
 Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 25 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 30 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 35 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 24 as follows:

20	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA	60
	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT-GCAAATTTCT-	120
	ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
	GGTGGAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG	240
	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
25	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTCTGA ACGCGCTGAA CGATATGTTA GCGGTTTCGC TGAACACGCT GGGCTCGAAA	420
	GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAACT	480
	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
	CCGATGCAGC AGCTGCTGAA GATGTTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
30	CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
	GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840



- 11 -

TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT 900  
 ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG 960  
 GCGAAGGAAA TCGGTCAAGT CATGGACCAG TATCCTGAGG TGTTCGGCAA GCCGCAGTAC 1020  
 CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAA AGCACTGAGC 1080  
 5 AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC 1140  
 ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC 1200  
 GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA 1260  
 CTTGGCAAGC TGGGCGCGGC TTAAGCTT 1288

10

Another potentially suitable hypersensitive response elicitor from  
*Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927,  
 which is hereby incorporated by reference. The protein is encoded by a DNA  
 molecule having a nucleic acid sequence of SEQ. ID. No. 25 as follows:

15 ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCTCGC CGGGTCTGTT CCAGTCCGGG 60  
 GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT 120  
 20 CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG 180  
 CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT 240  
 AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT 300  
 25 CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC 360  
 CAGGGCGGCG GGCAGATCGG CGATAATCCT TACTGAAAG CCATGCTGAA GCTTATTGCA 420  
 30 CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC 480  
 TCTTCCGGTA CTCTTCATC TGGCGGTTCC CCTTTAAGC ATCTATCAGG GGGGAAGGCC 540  
 CCTTCCGGCA ACTCCCCTTC CGGCAACTAC TCTCCCGTCA GTACCTTCTC ACCCCCATCC 600  
 35 ACGCCAACGT CCCCTACCTC ACGCTTGAT TTCCCTTCTT CTCCCACCA AGCAGCCGGG 660  
 GGCAGCACGC CGGTAACCGA TCATCCTGAC CTTGTTGGTA GCGCGGGCAT CGGGGCCGGA 720  
 40 AATTCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC 780  
 GTGAAAGCGG GTCAGGTGTT TGATGGCAA GGACAAACCT TCACCGCGG TTCAGAATTA 840  
 GCGGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CGGTGCCAGC 900  
 45 CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC 960  
 AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC 1020  
 50 AGCGCGGGCA AAAATCCCA CGTTGAAATC ACTAACAGTT CTTTCGAGCA CGCCTCTGAC 1080  
 AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC 1140

- 12 -

TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC 1200  
 CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC 1260  
 5 GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC 1320  
 GCCAACCTGA AGGTGGCTGA ATGA 1344

10

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 26 as follows:

15 Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu  
 1 5 10 15  
 Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser  
 20 20 25 30  
 Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala  
 35 40 45  
 25 Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly  
 50 55 60  
 Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly  
 65 70 75 80  
 30 Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro  
 85 90 95  
 Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu  
 100 105 110  
 35 Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp  
 115 120 125  
 Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp  
 40 130 135 140  
 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala  
 145 150 155 160  
 45 Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
 165 170 175  
 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
 180 185 190  
 50 Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro  
 195 200 205  
 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
 210 215 220  
 55

- 13 -

	Val	Thr	Asp	His	Pro	Asp	Pro	Val	Gly	Ser	Ala	Gly	Ile	Gly	Ala	Gly	225	230	235	240
5	Asn	Ser	Val	Ala	Phe	Thr	Ser	Ala	Gly	Ala	Asn	Gln	Thr	Val	Leu	His	245	250	255	
	Asp	Thr	Ile	Thr	Val	Lys	Ala	Gly	Gln	Val	Phe	Asp	Gly	Lys	Gly	Gln	260	265	270	
10	Thr	Phe	Thr	Ala	Gly	Ser	Glu	Leu	Gly	Asp	Gly	Gly	Gln	Ser	Glu	Asn	275	280	285	
	Gln	Lys	Pro	Leu	Phe	Ile	Leu	Glu	Asp	Gly	Ala	Ser	Leu	Lys	Asn	Val	290	295	300	
15	Thr	Met	Gly	Asp	Asp	Gly	Ala	Asp	Gly	Ile	His	Leu	Tyr	Gly	Asp	Ala	305	310	315	320
	Lys	Ile	Asp	Asn	Leu	His	Val	Thr	Asn	Val	Gly	Glu	Asp	Ala	Ile	Thr	325	330	335	
20	Val	Lys	Pro	Asn	Ser	Ala	Gly	Lys	Lys	Ser	His	Val	Glu	Ile	Thr	Asn	340	345	350	
	Ser	Ser	Phe	Glu	His	Ala	Ser	Asp	Lys	Ile	Leu	Gln	Leu	Asn	Ala	Asp	355	360	365	
	Thr	Asn	Leu	Ser	Val	Asp	Asn	Val	Lys	Ala	Lys	Asp	Phe	Gly	Thr	Phe	370	375	380	
30	Val	Arg	Thr	Asn	Gly	Gly	Gln	Gln	Gly	Asn	Trp	Asp	Leu	Asn	Leu	Ser	385	390	395	400
	His	Ile	Ser	Ala	Glu	Asp	Gly	Lys	Phe	Ser	Phe	Val	Lys	Ser	Asp	Ser	405	410	415	
35	Glu	Gly	Leu	Asn	Val	Asn	Thr	Ser	Asp	Ile	Ser	Leu	Gly	Asp	Val	Glu	420	425	430	
	Asn	His	Tyr	Lys	Val	Pro	Met	Ser	Ala	Asn	Leu	Lys	Val	Ala	Glu	435	440	445		

45 This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

50 Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663 which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 27 as follows:

- 14 -

	ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAC	60
	CCTGTGGGGC ATGGTGTTC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC	120
5	GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA	180
	TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG	240
10	GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC	300
	CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT	360
	GAGGCGGCCG CGCCAGATGC GCGCGTTTTG ACCCGTTTCG GCGGCGTCAA ACGCCGCAAT	420
15	ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA	480
	ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC	540
20	AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCGATCGCC TGCAGCATTC ACCGCCGCAC	600
	ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA	660
	ACGGCCACAG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
25	CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC	780
	GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAACTGA CTGCGGTTGC GGAAAGCGTC	840
30	CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
	GGAGCCGGGG TAACGCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG	960
	GATAATCCAC CCGCGCTCAA TACGTGTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC	1020
35	TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
	CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
40	GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAA	1200
	AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAACAAT GCTAAGCCAA	1260
	CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTGGC AGCATCCTGC TGGCGCAGCG	1320
45	CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG	1380
	CTGGGCGTAT GGCAATCTGC GGATAAGAT ACCCAGAGCC AGCTGTCTCG CCAGGCAGAC	1440
50	GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC	1500
	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG	1560
	ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC	1620
55	CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC	1680
	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTGTTGGCC	1740
60	GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAAGT	1800
	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT	1860
	TCTGGATTTT TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTGAAAAA TAACTTCAGG	1920
65	CAGCAGCATG CCTGCCCCTT GGGTAACGAT CATCAGTTTC ACCCGGCTG GAACCTGACT	1980

- 15 -

	GATGCGCTGG	TTATCGACAA	TCAGCTGGGG	CTGCATCATA	CCAATCCTGA	ACCGCATGAG	2040	
5	ATTCTTGATA	TGGGGCATT	AGGCAGCCTG	GCGTTACAGG	AGGGCAAGCT	TCACTATTTT	2100	
	GACCAGCTGA	CCAAAGGGTG	GACTGGCGCG	GAGTCAGATT	GTAAGCAGCT	GAAAAAAGGC	2160	
	CTGGATGGAG	CAGCTTATCT	ACTGAAAGAC	GGTGAAGTGA	AACGCCTGAA	TATTAATCAG	2220	
10	AGCACCTCCT	CTATCAAGCA	CGGAACGGAA	AACGTTTTTT	CGCTGCCGCA	TGTGCGCAAT	2280	
	AAACCGGAGC	CGGGAGATGC	CCTGCAAGGG	CTGAATAAAG	ACGATAAGGC	CCAGGCCATG	2340	
15	GCGGTGATTG	GGGTAAATAA	ATACCTGGCG	CTGACGGAAA	AAGGGGACAT	TCGCTCCTTC	2400	
	CAGATAAAAC	CCGGCACCCA	GCAGTTGGAG	CGGCCGGCAC	AAACTCTCAG	CCGCGAAGGT	2460	
	ATCAGCGGCG	AACTGAAAGA	CATTTCATGTC	GACCACAAGC	AGAACCTGTA	TGCCTTGACC	2520	
20	CACGAGGGAG	AGGTGTTTCA	TCAGCCGCGT	GAAGCCTGGC	AGAATGGTGC	CGAAAGCAGC	2580	
	AGCTGGCACA	AACTGGCGTT	GCCACAGAGT	GAAAGTAAGC	TAAAAAGTCT	GGACATGAGC	2640	
25	CATGAGCACA	AACCGATTGC	CACCTTTGAA	GACGGTAGCC	AGCATCAGCT	GAAGGCTGGC	2700	
	GGCTGGCACG	CCTATGCGGC	ACCTGAACGC	GGGCCGCTGG	CGGTGGGTAC	CAGCGGTTCA	2760	
	CAAACCGTCT	TTAACCGACT	AATGCAGGGG	GTGAAAGGCA	AGGTGATCCC	AGGCAGCGGG	2820	
30	TTGACGGTTA	AGCTCTCGGC	TCAGACGGGG	GGAATGACCG	GCGCCGAAGG	GCGCAAGGTC	2880	
	AGCAGTAAAT	TTTCCGAAAG	GATCCGCGCC	TATGCGTTCA	ACCCAACAAT	GTCCACGCCG	2940	
35	CGACCGATTA	AAAATGCTGC	TTATGCCACA	CAGCACGGCT	GGCAGGGGCG	TGAGGGGTTG	3000	
	AAGCCGTTGT	ACGAGATGCA	GGGAGCGCTG	ATTAAACAAC	TGGATGCGCA	TAACGTTCGT	3060	
	CATAACGCGC	CACAGCCAGA	TTTGCAGAGC	AAACTGGAAA	CTCTGGATTT	AGGCGAACAT	3120	
40	GGCGCAGAAT	TGCTTAACGA	CATGAAGCGC	TTCCGCGACG	AACTGGAGCA	GAGTGCAACC	3180	
	CGTTCGGTGA	CCGTTT	TAGG	TCAACATCAG	GGAGTGCTAA	AAAGCAACGG	TGAAATCAAT	3240
45	AGCGAATTTA	AGCCATCGCC	CGGCAAGGCG	TTGGTCCAGA	GCTTTAACGT	CAATCGCTCT	3300	
	GGTCAGGATC	TAAGCAAGTC	ACTGCAACAG	GCAGTACATG	CCACGCCGCC	ATCCGCAGAG	3360	
	AGTAAACTGC	AATCCATGCT	GGGGCACTTT	GTCAGTGCCG	GGGTGGATAT	GAGTCATCAG	3420	
50	AAGGGCGAGA	TCCCGCTGGG	CCGCCAGCGC	GATCCGAATG	ATAAAACCGC	ACTGACCAAA	3480	
	TCGCGTTTAA	TTTTAGATAC	CGTGACCATC	GGTGAAGTGC	ATGAACTGGC	CGATAAGGCG	3540	
55	AAACTGGTAT	CTGACCATAA	ACCCGATGCC	GATCAGATAA	AACAGCTGCG	CCAGCAGTTC	3600	
	GATACGCTGC	GTGAAAAGCG	GTATGAGAGC	AATCCGGTGA	AGCATTACAC	CGATATGGGC	3660	
	TTCACCCATA	ATAAGGCGCT	GGAAGCAAAC	TATGATGCGG	TCAAAGCCTT	TATCAATGCC	3720	
60	TTTAAGAAAG	AGCACCACGG	CGTCAATCTG	ACCACGCGTA	CCGTACTGGA	ATCACAGGGC	3780	
	AGTGCGGAGC	TGGCGAAGAA	GCTCAAGAAT	ACGCTGTTGT	CCCTGGACAG	TGGTGAAAGT	3840	
65	ATGAGCTTCA	GCCGGTCATA	TGGCGGGGGC	GTCAGCACTG	TCTTTGTGCC	TACCCTTAGC	3900	

- 16 -

	AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC	3960
	CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG	4020
5	AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
	ACCAGTGCAG GTAACGCCAG TGA CTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGA CTG	4140
10	CGTATCGGCG CTGCTGTGAG TGGCACCCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
	AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
	CCGGCAGAAC TGTTCGAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG	4320
15	TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC	4380
	GGCAGTAAAC CAAATGGTGT CACTGCCCCG GTTTCTGCCG GGCTAAGTGC ATCGGCAAAC	4440
20	CTGGCCGCGG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG	4500
	GCCAGCAATA ACCGCCCAAC CTTCCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT	4560
	GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAAC CGGTCGGGAT CTCCCGGCA	4620
25	TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
	AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740
30	ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG	4800
	TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTTAC AGCAGCATTT CAGTGCAAAA	4860
	GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT	4920
35	CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC	4980
	AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTGAGC TGCTACACAA ACATTTGAT	5040
40	GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA	5100
	CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCGATG	5160
	GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT	5220
45	CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TGCGTGTTAA ATCGGTCAGC	5280
	GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG	5340
50	AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC	5400
	CAGGATCAGA ACACCCACG GCGATTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG	5460
	CAGGTCGCAT CTGCGCTTAC TGATTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA	5517

55

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 28 as follows:

60

- 17 -

	Met	Glu	Leu	Lys	Ser	Leu	Gly	Thr	Glu	His	Lys	Ala	Ala	Val	His	Thr	
	1				5					10					15		
5	Ala	Ala	His	Asn	Pro	Val	Gly	His	Gly	Val	Ala	Leu	Gln	Gln	Gly	Ser	
				20					25					30			
	Ser	Ser	Ser	Ser	Pro	Gln	Asn	Ala	Ala	Ala	Ser	Leu	Ala	Ala	Glu	Gly	
				35				40					45				
10	Lys	Asn	Arg	Gly	Lys	Met	Pro	Arg	Ile	His	Gln	Pro	Ser	Thr	Ala	Ala	
		50					55					60					
	Asp	Gly	Ile	Ser	Ala	Ala	His	Gln	Gln	Lys	Lys	Ser	Phe	Ser	Leu	Arg	
15		65				70					75					80	
	Gly	Cys	Leu	Gly	Thr	Lys	Lys	Phe	Ser	Arg	Ser	Ala	Pro	Gln	Gly	Gln	
					85				90					95			
20	Pro	Gly	Thr	Thr	His	Ser	Lys	Gly	Ala	Thr	Leu	Arg	Asp	Leu	Leu	Ala	
				100					105					110			
	Arg	Asp	Asp	Gly	Glu	Thr	Gln	His	Glu	Ala	Ala	Ala	Pro	Asp	Ala	Ala	
				115				120					125				
25	Arg	Leu	Thr	Arg	Ser	Gly	Gly	Val	Lys	Arg	Arg	Asn	Met	Asp	Asp	Met	
		130					135					140					
	Ala	Gly	Arg	Pro	Met	Val	Lys	Gly	Gly	Ser	Gly	Glu	Asp	Lys	Val	Pro	
30		145				150					155					160	
	Thr	Gln	Gln	Lys	Arg	His	Gln	Leu	Asn	Asn	Phe	Gly	Gln	Met	Arg	Gln	
					165				170					175			
35	Thr	Met	Leu	Ser	Lys	Met	Ala	His	Pro	Ala	Ser	Ala	Asn	Ala	Gly	Asp	
				180					185					190			
	Arg	Leu	Gln	His	Ser	Pro	Pro	His	Ile	Pro	Gly	Ser	His	His	Glu	Ile	
			195					200					205				
40	Lys	Glu	Glu	Pro	Val	Gly	Ser	Thr	Ser	Lys	Ala	Thr	Thr	Ala	His	Ala	
		210					215					220					
	Asp	Arg	Val	Glu	Ile	Ala	Gln	Glu	Asp	Asp	Asp	Ser	Glu	Phe	Gln	Gln	
45		225				230				235						240	
	Leu	His	Gln	Gln	Arg	Leu	Ala	Arg	Glu	Arg	Glu	Asn	Pro	Pro	Gln	Pro	
					245				250					255			
50	Pro	Lys	Leu	Gly	Val	Ala	Thr	Pro	Ile	Ser	Ala	Arg	Phe	Gln	Pro	Lys	
				260				265						270			
	Leu	Thr	Ala	Val	Ala	Glu	Ser	Val	Leu	Glu	Gly	Thr	Asp	Thr	Thr	Gln	
				275				280					285				
55	Ser	Pro	Leu	Lys	Pro	Gln	Ser	Met	Leu	Lys	Gly	Ser	Gly	Ala	Gly	Val	
		290				295						300					
	Thr	Pro	Leu	Ala	Val	Thr	Leu	Asp	Lys	Gly	Lys	Leu	Gln	Leu	Ala	Pro	
60		305				310					315					320	
	Asp	Asn	Pro	Pro	Ala	Leu	Asn	Thr	Leu	Leu	Lys	Gln	Thr	Leu	Gly	Lys	
					325				330					335			
65	Asp	Thr	Gln	His	Tyr	Leu	Ala	His	His	Ala	Ser	Ser	Asp	Gly	Ser	Gln	
				340				345						350			

- 18 -

His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr  
 355 360 365  
 5 Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys  
 370 375 380  
 Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys  
 385 390 395 400  
 10 Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr  
 405 410 415  
 15 Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile  
 420 425 430  
 Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg  
 435 440 445  
 20 Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp  
 450 455 460  
 Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp  
 465 470 475 480  
 25 Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser  
 485 490 495  
 30 Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser  
 500 505 510  
 Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg  
 515 520 525  
 35 His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His  
 530 535 540  
 Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His  
 545 550 555 560  
 40 Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg  
 565 570 575  
 45 Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro  
 580 585 590  
 Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His  
 595 600 605  
 50 Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe  
 610 615 620  
 His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg  
 625 630 635 640  
 55 Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly  
 645 650 655  
 60 Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His  
 660 665 670  
 His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly  
 675 680 685



- 19 -

	Ser	Leu	Ala	Leu	Gln	Glu	Gly	Lys	Leu	His	Tyr	Phe	Asp	Gln	Leu	Thr	
	690						695					700					
5	Lys	Gly	Trp	Thr	Gly	Ala	Glu	Ser	Asp	Cys	Lys	Gln	Leu	Lys	Lys	Gly	
	705					710					715					720	
	Leu	Asp	Gly	Ala	Ala	Tyr	Leu	Leu	Lys	Asp	Gly	Glu	Val	Lys	Arg	Leu	
					725					730					735		
10	Asn	Ile	Asn	Gln	Ser	Thr	Ser	Ser	Ile	Lys	His	Gly	Thr	Glu	Asn	Val	
				740					745					750			
	Phe	Ser	Leu	Pro	His	Val	Arg	Asn	Lys	Pro	Glu	Pro	Gly	Asp	Ala	Leu	
			755					760					765				
15	Gln	Gly	Leu	Asn	Lys	Asp	Asp	Lys	Ala	Gln	Ala	Met	Ala	Val	Ile	Gly	
		770					775					780					
20	Val	Asn	Lys	Tyr	Leu	Ala	Leu	Thr	Glu	Lys	Gly	Asp	Ile	Arg	Ser	Phe	
	785					790					795					800	
	Gln	Ile	Lys	Pro	Gly	Thr	Gln	Gln	Leu	Glu	Arg	Pro	Ala	Gln	Thr	Leu	
					805					810					815		
25	Ser	Arg	Glu	Gly	Ile	Ser	Gly	Glu	Leu	Lys	Asp	Ile	His	Val	Asp	His	
				820					825					830			
	Lys	Gln	Asn	Leu	Tyr	Ala	Leu	Thr	His	Glu	Gly	Glu	Val	Phe	His	Gln	
			835					840					845				
30	Pro	Arg	Glu	Ala	Trp	Gln	Asn	Gly	Ala	Glu	Ser	Ser	Ser	Trp	His	Lys	
		850					855					860					
35	Leu	Ala	Leu	Pro	Gln	Ser	Glu	Ser	Lys	Leu	Lys	Ser	Leu	Asp	Met	Ser	
	865					870					875					880	
	His	Glu	His	Lys	Pro	Ile	Ala	Thr	Phe	Glu	Asp	Gly	Ser	Gln	His	Gln	
					885					890					895		
40	Leu	Lys	Ala	Gly	Gly	Trp	His	Ala	Tyr	Ala	Ala	Pro	Glu	Arg	Gly	Pro	
				900					905					910			
	Leu	Ala	Val	Gly	Thr	Ser	Gly	Ser	Gln	Thr	Val	Phe	Asn	Arg	Leu	Met	
			915					920					925				
45	Gln	Gly	Val	Lys	Gly	Lys	Val	Ile	Pro	Gly	Ser	Gly	Leu	Thr	Val	Lys	
		930					935					940					
50	Leu	Ser	Ala	Gln	Thr	Gly	Gly	Met	Thr	Gly	Ala	Glu	Gly	Arg	Lys	Val	
	945					950					955					960	
	Ser	Ser	Lys	Phe	Ser	Glu	Arg	Ile	Arg	Ala	Tyr	Ala	Phe	Asn	Pro	Thr	
					965					970					975		
55	Met	Ser	Thr	Pro	Arg	Pro	Ile	Lys	Asn	Ala	Ala	Tyr	Ala	Thr	Gln	His	
				980					985					990			
	Gly	Trp	Gln	Gly	Arg	Glu	Gly	Leu	Lys	Pro	Leu	Tyr	Glu	Met	Gln	Gly	
			995					1000					1005				
60	Ala	Leu	Ile	Lys	Gln	Leu	Asp	Ala	His	Asn	Val	Arg	His	Asn	Ala	Pro	
		1010					1015					1020					
65	Gln	Pro	Asp	Leu	Gln	Ser	Lys	Leu	Glu	Thr	Leu	Asp	Leu	Gly	Glu	His	
	1025					1030					1035					1040	

- 20 -

Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu  
 1045 1050 1055  
 5 Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val  
 1060 1065 1070  
 Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly  
 1075 1080 1085  
 10 Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu  
 1090 1095 1100  
 Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu  
 1105 1110 1115 1120  
 15 Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp  
 1125 1130 1135  
 20 Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro  
 1140 1145 1150  
 Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val  
 1155 1160 1165  
 25 Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser  
 1170 1175 1180  
 Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe  
 1185 1190 1195 1200  
 30 Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr  
 1205 1210 1215  
 35 Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp  
 1220 1225 1230  
 Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val  
 1235 1240 1245  
 40 Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu  
 1250 1255 1260  
 Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser  
 1265 1270 1275 1280  
 45 Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val  
 1285 1290 1295  
 50 Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly  
 1300 1305 1310  
 Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly  
 1315 1320 1325  
 55 Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile  
 1330 1335 1340  
 Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys  
 1345 1350 1355 1360  
 60 Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile  
 1365 1370 1375

- 21 -

Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly  
 1380 1385 1390  
 5 Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro  
 1395 1400 1405  
 Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu  
 1410 1415 1420  
 10 Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr  
 1425 1430 1435 1440  
 Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn  
 1445 1450 1455  
 15 Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser  
 1460 1465 1470  
 20 Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg  
 1475 1480 1485  
 Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn  
 1490 1495 1500  
 25 Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala  
 1505 1510 1515 1520  
 Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly  
 1525 1530 1535  
 30 Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu  
 1540 1545 1550  
 35 Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu  
 1555 1560 1565  
 Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys  
 1570 1575 1580  
 40 His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu  
 1585 1590 1595 1600  
 Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His  
 1605 1610 1615  
 45 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg  
 1620 1625 1630  
 50 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser  
 1635 1640 1645  
 Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser  
 1650 1655 1660  
 55 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp  
 1665 1670 1675 1680  
 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn  
 1685 1690 1695  
 60 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro  
 1700 1705 1710  
 65 Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu  
 1715 1720 1725

- 22 -

5           Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val  
               1730                       1735                       1740  
           Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser  
               1745                       1750                       1755                       1760  
           Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu  
                                   1765                       1770                       1775  
 10           Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile  
                                   1780                       1785                       1790  
           Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg  
                                   1795                       1800                       1805  
 15           Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser  
                                   1810                       1815                       1820  
 20           Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser  
                                   1825                       1830                       1835

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

25           The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 29 as follows:

ATGACATCGT CACAGCAGCG GGTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAG           60  
 30   ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG           120  
       GCGGTGCTGG AAGTACCGCA ACAAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG           180  
       GCTGACCCAC AAACCTCAAT AACCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG           240  
 35   GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT           300  
       CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC           360  
 40   GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA           420

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 30 as follows:

50           Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser  
               1                       5                       10                       15  
           Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu  
                                   20                       25                       30  
           Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His  
                                   35                       40                       45  
 55           Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln  
                                   50                       55                       60

- 23 -

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met  
 65 70 75 80  
 5 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val  
 85 90 95  
 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe  
 100 105 110  
 10 Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu  
 115 120 125  
 Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala  
 130 135  
 15

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.

20 No. 31 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1 5 10 15  
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
 20 25 30  
 25 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
 35 40 45  
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
 50 55 60  
 30 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
 65 70 75 80  
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85 90 95  
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100 105 110  
 35 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125  
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140  
 40 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160  
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175  
 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
 180 185 190

- 24 -

	Gly	Gln	Gln	Leu	Gly	Asn	Gln	Gln	Ser	Asp	Ala	Gly	Ser	Leu	Ala	Gly	
				195				200					205				
	Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser	
		210					215						220				
5	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser	
		225				230					235					240	
	Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp	
					245					250					255		
10	Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val	
				260					265					270			
	Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln	
			275					280					285				
	Asp	Leu	Asp	Gln	Leu	Leu	Gly	Gly	Leu	Leu	Leu	Lys	Gly	Leu	Glu	Ala	
		290					295					300					
15	Thr	Leu	Lys	Asp	Ala	Gly	Gln	Thr	Gly	Thr	Asp	Val	Gln	Ser	Ser	Ala	
		305				310					315					320	
	Ala	Gln	Ile	Ala	Thr	Leu	Leu	Val	Ser	Thr	Leu	Leu	Gln	Gly	Thr	Arg	
					325					330					335		
20	Asn	Gln	Ala	Ala	Ala												
					340												

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine.

- 25 Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas syringae pv. syringae Harpin<sub>PS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the
- 30 hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 32 as follows:

	ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTTG	60
	GTACGTCTCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTG	120
35	GTGAAGCTGG CCGAGGAAGT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA	180
	AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC	240
	ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG	300

- 25 -

GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC 360  
 AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420  
 GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480  
 AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540  
 5 GAAACGGCTG CGTTCCGTTC GGCAC TCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600  
 AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660  
 AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720  
 GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780  
 TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCGCAGAC CGGTACGTCG 840  
 10 GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900  
 GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960  
 GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020  
 GCCTGA 1026

15 Another potentially suitable hypersensitive response elicitor from  
*Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817,  
 which is hereby incorporated by reference. The protein has a nucleotide sequence of  
 SEQ. ID. No. 33 as follows:

20 TCCAATTGCG TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG 60  
 CTGAGTGC GC AGATTTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAAGG 120  
 CCTCTGAGTG CCGTGCGGAG CAATACCAGT CTTCTGCTG GCGTGTGCAC ACTGAGTCGC 180  
 25 AGGCATAGGC ATTTTCAGTTC CTTGCGTTGG TTGGGCATAT AAAAAAAGGA ACTTTTAAAA 240  
 ACAGTGCAAT GAGATGCCCG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG 300  
 30 AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC 360  
 TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCCAC TTAGCGAGGT AACGCAGCAT 420  
 GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCACG CCACTCGATT TTTCGGCGCT 480  
 35 AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA 540  
 CCCGAGTGCA CTGTTGTTCTG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCCGA 600  
 40 CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC 660  
 TAAATTGATC AGTGCAATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA 720  
 GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT 780

- 26 -

CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG 840  
 5 CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG 900  
 TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC 960  
 CACTGCAACA GGC GTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA 1020  
 10 CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC 1080  
 CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG 1140  
 CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA 1200  
 15 GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA 1260  
 CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT 1320  
 20 GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT 1380  
 CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT 1440  
 CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTCTG GCACGATGGT 1500  
 25 TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC 1560  
 TAACCACGGC AAGTTCGCCC TGGTGAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG 1620  
 30 CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680  
 CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC 1729

35 This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 34 as follows:

40 Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu  
 1 5 10 15  
 Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly  
 20 25 30  
 45 Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly  
 35 40 45  
 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val  
 50 50 55 60  
 Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile  
 65 70 75 80  
 55 Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr  
 85 90 95



- 27 -

	Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln	100	105	110
5	Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser	115	120	125
	Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr	130	135	140
10	Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly	145	150	155
	Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly	165	170	175
15	Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr	180	185	190
20	Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr	195	200	205
	Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile	210	215	220
25	Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp	225	230	235
	Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp	245	250	255
30	Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr	260	265	270
35	Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val	275	280	285
	Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln	290	295	300
40	Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala	305	310	315
	Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp	325	330	335
45	Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe	340	345	350
50	Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln	355	360	365
	Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly	370	375	380
55	Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr	385	390	395
	Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln	405	410	415

- 28 -

Ala Ser Thr Gln His Thr Glu Leu  
420

5

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.

10 ID. No. 35 as follows:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln  
1 5 10 15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser  
15 20 25 30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile  
35 40 45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly  
50 55 60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala  
20 65 70 75 80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser  
85 90 95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met  
25 100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val  
130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
30 145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly  
35 180 185 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
210 215 220

- 29 -

	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp
	225					230					235					240
	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn
					245					250					255	
5	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln
				260					265					270		
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly
			275					280					285			
10	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser
		290					295					300				
	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val
	305					310					315					320
	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln
					325				330						335	
15	Gln	Ser	Thr	Ser	Thr	Gln	Pro	Met								
					340											

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.  
ID. No. 36 as follows:

	ATGTCAGTCG	GAAACATCCA	GAGCCCGTCG	AACCTCCCGG	GTCTGCAGAA	CCTGAACCTC	60
20	AACACCAACA	CCAACAGCCA	GCAATCGGGC	CAGTCCGTGC	AAGACCTGAT	CAAGCAGGTC	120
	GAGAAGGACA	TCCTCAACAT	CATCGCAGCC	CTCGTGCAGA	AGGCCGCACA	GTCCGGCGGC	180
	GGCAACACCG	GTAACACCGG	CAACGCGCCG	GCGAAGGACG	GCAATGCCAA	CGCGGGCGCC	240
	AACGACCCGA	GCAAGAACGA	CCCGAGCAAG	AGCCAGGCTC	CGCAGTCGGC	CAACAAGACC	300
	GGCAACGTCG	ACGACGCCAA	CAACCAGGAT	CCGATGCAAG	CGCTGATGCA	GCTGCTGGAA	360
25	GACCTGGTGA	AGCTGCTGAA	GGCGGCCCTG	CACATGCAGC	AGCCGGGCGG	CAATGACAAG	420
	GGCAACGGCG	TGGGCGGTGC	CAACGGCGCC	AAGGGTGCCG	GCGGCCAGGG	CGGCCTGGCC	480
	GAAGCGCTGC	AGGAGATCGA	GCAGATCCTC	GCCCAGCTCG	GCGGCGGCGG	TGCTGGCGCC	540
	GGCGGCGCGG	GTGGCGGTGT	CGGCGGTGCT	GGTGGCGCGG	ATGGCGGCTC	CGGTGCGGGT	600
	GGCGCAGGCG	GTGCGAACGG	CGCCGACGGC	GGCAATGGCG	TGAACGGCAA	CCAGGCGAAC	660
30	GGCCCGCAGA	ACGCAGGCGA	TGTCAACGGT	GCCAACGGCG	CGGATGACGG	CAGCGAAGAC	720
	CAGGGCGGCC	TCACCGGCGT	GCTGCAAAAG	CTGATGAAGA	TCCTGAACGC	GCTGGTGCAG	780
	ATGATGCAGC	AAGGCGGCCT	CGGCGGCGGC	AACCAGGCGC	AGGGCGGCTC	GAAGGGTGCC	840
	GGCAACGCCT	CGCGGGCTTC	CGGCGCGAAC	CCGGGCGCGA	ACCAGCCCGG	TTCGGCGGAT	900

- 30 -

GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC 960  
 GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG 1020  
 ACGCAGCCGA TGTAA 1035

5

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

10

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 37 as follows:

15

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala  
 1 5 10 15  
 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr  
 20 25

20

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

25

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease-sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 38 as follows:

30

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln  
 1 5 10 15  
 Leu Leu Ala Met  
 20

35

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

- 31 -

subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not  
5 Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from  
10 *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and  
15 Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A  
20 Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

25 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by  
30 growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture

- 32 -

supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia amylovora* hypersensitive response elicitor. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, or an internal fragment of the amino acid sequence of SEQ. ID. No. 23. The C-terminal fragment of the amino acid

- 33 -

sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 23 can span the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable  
5 fragments can be identified in accordance with the present invention.

Another example of a useful fragment of a hypersensitive response elicitor which fragment does not itself elicit a hypersensitive response is the protein fragment containing amino acids 190 to 294 of the amino acid sequence (SEQ. ID. No. 31) for the *Pseudomonas syringae* pv. *syringae* hypersensitive response elicitor.  
10 This fragment is useful in imparting disease resistance and enhancing plant growth.

Yet another example of a useful fragment of a hypersensitive response elicitor is the peptide having an amino acid sequence corresponding to SEQ. ID. No. 39. This peptide is derived from the hypersensitive response eliciting glycoprotein of *Phytophthora megasperma* and enhances plant growth.

15 Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide  
20 may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The fragment of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional  
25 techniques. Typically, the fragment of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein fragment, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment,  
30 and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the fragment is separated by centrifugation. The supernatant fraction containing the fragment is subjected to gel filtration in an

- 34 -

appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A



- 35 -

Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

- 36 -

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the  $P_R$  and  $P_L$  promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

- 37 -

Once the isolated DNA molecule encoding the fragment of a hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, and/or effecting insect control for plants. These methods involve applying the fragment of a hypersensitive response elicitor polypeptide or protein which does not elicit a hypersensitive response in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the fragment to impart disease resistance, enhance growth, and/or control insects. Alternatively, these fragments of a hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, and/or to effect insect control.

As an alternative to applying a fragment of a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, and/or to control insects on the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, and/or to control insects.

- 38 -

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated fragment or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the fragment. In the latter embodiment, the fragment can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the fragment of the hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response. Such bacteria must be capable of secreting or exporting the fragment so that the fragment can contact plant or plant seed cells. In these embodiments, the fragment is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, and/or control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the fragments of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of

- 39 -

pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention:

- 5 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

- With regard to the use of the fragments of the hypersensitive response
- 10 elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds
- 15 germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed
- 20 germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

- Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present
- 25 invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from
- 30 releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

- 40 -

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding  
5 pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect  
10 pests represents the most economically important group of pests for vegetable production worldwide.

The method of the present invention involving application of the fragment of a hypersensitive response elicitor polypeptide or protein, which fragment does not elicit a hypersensitive response, can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots,  
15 propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the fragment of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of  
20 the present invention, the fragment of the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the fragment with cells of the plant or plant seed. Once treated with  
25 the fragment of the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the fragment of the hypersensitive response elicitor protein or  
30 polypeptide or whole elicitors to impart disease resistance to plants, to enhance plant growth, and/or to control insects on the plants.

- 41 -

The fragment of the hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the fragment can be applied separately to plants with other materials being applied at different times.

5 A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a fragment of a hypersensitive response elicitor polypeptide or protein which fragment does not elicit a hypersensitive response in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains  
10 greater than 500 nM of the fragment.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

15 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response eliciting fragment can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

20 In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a fragment of a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such a fragment are produced according to procedures well known in the art.

25 The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby  
30 incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic

transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy



- 43 -

root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated. Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

- 44 -

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves  
5 can be cultivated in accordance with conventional procedure with the presence of the gene encoding the fragment of the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, and/or control of insects on the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using  
10 conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, and/or to control insects. While not wishing to be bound by theory, such disease resistance, growth enhancement, and/or insect control may be RNA mediated or may result from expression of the  
15 polypeptide or protein fragment.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a fragment of a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including  
20 a fragment of a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the fragment of a  
25 hypersensitive response elicitor in accordance with the present invention to impart disease resistance, enhance growth, and/or control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

## EXAMPLES

30

### Example 1 - Bacterial Strains and Plasmids

*Escherichia coli* strains used in the following examples include DH5 $\alpha$  and BL21(DE3) purchased from Gibco BRL (Grand Island, N.Y.) and Stratagene

- 45 -

(La Jolla, CA), respectively. The pET28(b) vector was purchased from Novagen (Madison, WI). Eco DH5 $\alpha$ /2139 contained the complete *hrpN* gene. The 2139 construct was produced by D. Bauer at Cornell University. The *hrpN* gene was cleaved from the 2139 plasmid by restriction enzyme digestion with HindIII, then  
5 purified from an agarose gel to serve as the DNA template for PCR synthesis of truncated *hrpN* clones. These clones were subsequently inserted into the (His)<sub>6</sub> vector pET28(b) which contained a Kan<sup>r</sup> gene for selection of transformants.

### **Example 2 - DNA Manipulation**

10 Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, IN) or Gibco BRL. T4 DNA ligase, Calf Intestinal Alkaline Phosphatase (CIAP), and PCR Supermix<sup>TM</sup> were obtained from Gibco BRL. The QIAprep Spin Miniprep Kit, the Qiagen Plasmid Mini Kit, and the QIAquick PCR  
15 Purification Kit were purchased from Qiagen (Hilden, Germany). The PCR primers were synthesized by Lofstrand Labs Limited (Gaithersburg, MD). The oligopeptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX). All DNA manipulations such as plasmid isolation, restriction enzyme digestion, DNA ligation, and PCR were performed according to standard techniques (Sambrook, et al., Laboratory Manual,  
20 Second Edition, Cold Spring Harbor Laboratory Press (1989)) or protocols provided by the manufacturer.

### **Example 3 - Fragmentation of *hrpN* Gene**

25 A series of N-terminal and C-terminal truncated *hrpN* genes and internal fragments were generated via PCR (Fig. 1). The full length *hrpN* gene was used as the DNA template and 3' and 5' primers were designed for each truncated clone (Fig. 2). The 3' primers contained an NdeI enzyme cutting site which contained the start codon ATG (methionine) and the 5' primers contained the stop codon TAA  
30 and a HindIII enzyme cutting site for ligation into the pET28(b) vector. PCR was carried out in 0.5 ml tubes in a GeneAmp<sup>TM</sup> 9700 (Perkin-Elmer, Foster City, CA). 45  $\mu$ l of Supermix<sup>TM</sup> (Life Technology, Gaithersburg, MD) were mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and deionized

- 46 -

H<sub>2</sub>O to a final volume of 50 µl. After heating the mixture at 95°C for 2 min, the PCR was performed for 30 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1.5 min. The PCR products were verified on a 6% TBE gel (Novex, San Diego, CA).

Amplified DNA was purified with the QIAquick PCR purification kit, digested with

5 Nde I and Hind III at 37°C for 5 hours, extracted once with

phenol:chloroform:isoamylalcohol (25:25:1) and precipitated with ethanol. 5 µg of pET28(b) vector DNA were digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with CIAP treatment to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with

10 the QIAquick PCR purification kit and directly used for ligation. Ligation was carried out at 14-16°C for 5-12 hours in a 15 µl mixture containing ca. 200 ng of digested pET28(b), 30 ng of targeted PCR fragment, and 1 unit T4 DNA ligase. 5 - 7.5 µl of

ligation solution were added to 100 µl of DH5α competent cells in a 15 ml Falcon tube and incubated on ice for 30 min. After a heat shock at 42°C for 45 seconds, 0.9

15 ml SOC solution or 0.45 ml LB media were added to each tube and incubated at 37°C for 1 hour. 20, 100, and 200 µl of transformed cells were placed onto LB agar with 30 µg/ml of kanamycin and incubated at 37°C overnight. Single colonies were

transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared from 2 ml of culture with the QIAprep Miniprep kit (QIAGEN, Hilden,

20 Germany). The DNA from the transformed cells was analyzed by restriction enzyme digestion or partial sequencing to verify the success of the transformations. Plasmids

with the desired DNA sequence were transferred into the BL21 strain using the standard chemical transformation method as indicated above. A clone containing the full length harpin protein in the pET28(b) vector was generated as a positive control,

25 and a clone with only the pET28(b) vector was generated as a negative control.

#### **Example 4 - Expression of Hypersensitive Response Elicitor Truncated Proteins**

*Escherichia coli* BL21(DE3) strains containing the hrpN clones were  
30 grown in Luria broth medium (5g/L Difco Yeast extract, 10 g/L Difco Tryptone, 5 g/L NaCl, and 1 mM NaOH) containing 30 µg/ml of kanamycin at 37°C overnight. The bacteria were then inoculated into 100 volumes of the same medium and grown at

- 47 -

37°C to an OD<sub>620</sub> of 0.6-0.8. The bacteria were then inoculated into 250 volumes of the same medium and grown at 37°C to an OD<sub>620</sub> of ca. 0.3 or 0.6-0.8. One millimolar IPTG was then added and the cultures grown at 19°C overnight (ca. 18 hours). Not all of the clones were successfully expressed using this strategy. Several of the clones had to be grown in Terrific broth (12 g/L Bacto Tryptone, 24 g/L Bacto yeast, 0.4% glycerol, 0.17 M KH<sub>2</sub>PO<sub>4</sub>, and 0.72 K<sub>2</sub>HPO<sub>4</sub>), and/or grown at 37°C after IPTG induction, and/or harvested earlier than overnight (Table 1).

Table 1: Expression of hypersensitive response elicitor truncated proteins

Fragment	amino acids (SEQ. ID. No. 23)	Growth medium	Induction O.D.	Expression temp.	Harvest time
1 (+ control)	1-403	LB	ca. 0.3 or 0.6-0.8	19°C or 25°C	16-18 hr
2 (+ control)	-	LB and TB	ca. 0.3 or 0.6-0.8	19 C and 37 C	16-18 hr
3	105-403	LB	0.6-0.8	19°C	16-18 hr
4	169-403	TB	ca. 0.3	19°C	16-18 hr
5	210-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
6	257-403	LB or M9ZB	0.6-0.8	19°C	16-18 hr
7	343-403	LB	ca. 0.3	19°C	5 hr
8	1-75	TB	ca. 0.3	37°C	16-18 hr
9	1-104	TB	ca. 0.3	37°C	16-18 hr
10	1-168	TB	ca. 0.3	37°C	16-18 hr
11	1-266	LB	ca. 0.3	37°C	4 hr
12	1-342	LB	0.6-0.8	19°C	16-18 hr
13	76-209	LB	ca. 0.3	37°C	5 hr
14	76-168	TB or LB	ca. 0.3	37°C	3 hr or 16-18 hr
15	105-209	M9ZB	ca. 0.3	37°C	3 hr
16	169-209	no expression			
17	105-168	LB	ca. 0.3	37°C	3-5 hr
18	99-209	LB	ca. 0.3	37°C	3 hr
19	137-204	LB	ca. 0.3	37°C	3 hr
20	137-180	LB	ca. 0.3	37°C	16-18 hr.
21	105-180	LB	ca. 0.3	37°C	3 hr
22	150-209	no expression			
23	150-180	no expression			

#### Example 5 - Small Scale Purification of Hypersensitive Response Elicitor Truncated Proteins (Verification of Expression)

A 50 ml culture of a hrpN clone was grown as above to induce expression of the truncated protein. Upon harvesting of the culture, 1.5 ml of the cell

- 48 -

suspension were centrifuged at 14,000 rpm for 5 minutes, re-suspended in urea lysis buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.01 M Tris -- pH 8.0), incubated at room temperature for 10 minutes, then centrifuged again at 14,000 rpm for 10 minutes, and the supernatant saved. A 50 µl aliquot of a 50% slurry of an equilibrated (His)<sub>6</sub>-  
5 binding nickel agarose resin was added to the supernatant and mixed at 4°C for one hour. The nickel agarose was then washed three times with urea washing buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.01 M Tris -- pH 6.3), centrifuging at 5,000 rpm for five minutes between washings. The protein was eluted from the resin with 50 µl of urea elution buffer (8 M urea, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.01 M Tris, and 0.1 M EDTA -- pH 6.3).  
10 The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression.

#### **Example 6 - Induction of HR in Tobacco**

A 1.5 ml aliquot from the 50 ml cultures grown for small scale  
15 purification of the truncated proteins was centrifuged at 14,000 rpm for four minutes and re-suspended in an equal volume of 5 mM potassium phosphate buffer, pH 6.8. The cell suspension was sonicated for ca. 30 seconds then diluted 1:2 and 1:10 with phosphate buffer. Both dilutions plus the neat cell lysate were infiltrated into the fourth to ninth leaves of 10-15 leaf tobacco plants by making a hole in single leaf  
20 panes and infiltrating the bacterial lysate into the intercellular leaf space using a - - - - - syringe without a needle. The HR response was recorded 24-48 hr post infiltration. Tobacco (*Nicotiana tabacum* v. Xanthi) seedlings were grown in an environmental chamber at 20-25°C with a photoperiod of 12-h light /12-h dark and ca. 40% RH. Cell lysate was used for the initial HR assays (in order to screen the truncated proteins  
25 for HR activity) as the small scale urea purification yielded very little protein which was denatured due to the purification process.

#### **Example 7 - Large Scale Native Purification of Hypersensitive Response Elicitor Truncated Proteins for Comprehensive Biological Activity Assays**

30 Six 500 ml cultures of a hrpN clone were grown as described earlier to induce expression of the truncated protein. Upon harvesting of the culture, the cells were centrifuged at 7,000 rpm for 5 minutes, re-suspended in imidazole lysis buffer (5

- 49 -

mM imidazole, 0.5 M NaCl, 20 mM Tris) plus Triton X-100 at 0.05% and lysozyme at 0.1 mg/ml, incubated at 30°C for 15 minutes, sonicated for two minutes, centrifuged again at 15,000 rpm for 20 minutes, and the supernatant was saved. A 4 ml aliquot of a 50% slurry of an equilibrated (His)<sub>6</sub>-binding nickel agarose resin was added to the supernatant and mixed at 4°C for ca. four hours. The nickel agarose was then washed three times with imidazole washing buffer (20 mM imidazole, 0.5 M NaCl, and 20 mM Tris), centrifuging at 5,000 rpm for five minutes between washings, then placed in a disposable chromatography column. The column was centrifuged at 1100 rpm for one minute to remove any residual wash buffer and then the protein was eluted from the resin with 4 ml of imidazole elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris) by incubating the column with the elution buffer for ten minutes at room temperature and then centrifuging the column at 1100 rpm for one minute. The eluate was run on a 4-20%, a 16%, or a 10-20% Tris-Glycine pre-cast gel depending upon the size of the truncated protein to verify the expression. The concentration of the proteins was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker.

**Example 8 - Large Scale Urea Purification of Hypersensitive Response Elicitor Truncated Proteins For Comprehensive Biological Activity Assay**

The procedure was the same as the large scale native purification except that urea lysis buffer, washing buffer, and elution buffer were used, and the cells were not sonicated as in the native purification. After purification, the protein was renatured by dialyzing against lower and lower concentrations of urea over an eight hour period, then dialyzing overnight against 10 mM Tris/20 mM NaCl. The renaturing process caused the N-terminal proteins to precipitate. The precipitated 1-168 protein was solubilized by the addition of 100 mM Tris-HCl at pH 10.4 then heating the protein at 30°C for ca. one hour. The concentration of the protein was determined by comparison of the protein bands with a standard protein in the Mark 12 molecular weight marker. The 1-75 and 1-104 protein fragments were not successfully solubilized using this strategy so they were sonicated in 100 mM Tris-HCl at pH 10.4 to solubilize as much of the protein as possible and expose the active sites of the protein for the biological activity assays.

**Example 9 – Induction of Growth Enhancement (GE)**

Sixty tomato (*Lycopersicon spp.* cv. Marglobe) seeds were soaked overnight in 10 and 20 µg/ml of the truncated protein diluted with 5mM potassium phosphate buffer, pH 6.8. The next morning, the sixty seeds were sewn in three pots and 12-15 days later and again 18-20 days later the heights of the 10 tallest tomato plants per pot were measured and compared with the heights of the control plants treated only with phosphate buffer. Analyses were done on the heights to determine if there was a significant difference in the height of the plants treated with the truncated proteins compared with the buffer control, and thereby determine whether the proteins induced growth enhancement.

**Example 10 – Induction of Systemic Acquired Resistance (SAR)**

Three tobacco (*Nicotiana tabacum* cv. Xanthi) plants with 8-12 leaves (ca. 75 day old plants) were used in the assay. One leaf of the tobacco plants was covered up and the rest of the leaves were sprayed with ca. 50 ml of a 20 µg/ml solution of the truncated proteins diluted with 5mM potassium phosphate buffer. Five to seven days later two leaves (the unsprayed leaf and the sprayed leaf opposite and just above the unsprayed leaf) were inoculated with 20 µl of a 1.8 µg/ml solution of TMV along with a pinch of diatomaceous earth by rubbing the mixture along the top surface of the leaves. The TMV entered the plants through tiny lesions made by the diatomaceous earth. Ca. 3-4 days post TMV inoculation, the number of TMV lesions was counted on both leaves compared with the number of lesions on the negative control buffer treated leaves. Analyses were done to determine the efficacy of reducing the number of TMV lesions by the protein fragments compared to the buffer control. Percentage of efficacy was calculated as: Reduction in TMV lesions (% efficacy) = 100 x (1 – mean # of lesions on treated leaves/mean # of lesions on buffer control leaves).



- 51 -

**Example 11 - Expression of Hypersensitive Response Elicitor Truncated Proteins**

The small scale expression and purification of the fragment proteins was done to screen for expression and HR activity (Table 2).

5

Table 2

Expression and HR activity of hypersensitive response elicitor truncated proteins (small scale screening)

Fragment #	Amino Acids (SEQ. ID. No. 23)	Expression	HR activity
1(+control)	1-403	+	+
2(- control)	-	background protein only	-
3	105-403	+	+
4	169-403	+	-
5	210-403	+	-
6	267-403	+	-
7	343-403	+/-	-
8	1-75	+	-
9	1-104	+	+/-
10	1-168	+	+
11	1-266	+	+
12	1-342	+	+
13	76-209	+	+
14	76-168	+	-
15	105-209	+	+
16	169-209	-	-
17	105-168	+	-
18	99-209	+	+
19	137-204	+	+
20	137-180	+	+
21	105-180	+	+
22	150-209	-	-
23	150-180	-	-

10

All of the cloned fragment proteins were expressed at varying levels except for three small fragments (amino acids 169-209, 150-209, and 150-180). Fragments 210-403 and 267-403 were expressed very well, yielding a high concentration of protein from a small scale purification, resulting in a substantial protein band on SDS gel electrophoresis. Other fragments (such as a.a. 1-168 and 1-104) produced much less protein, resulting in faint protein bands upon electrophoresis. It was difficult to determine whether fragment 343-403, the smallest C-terminal protein, was expressed, as there were several background proteins apparent on the gel, in addition to the suspected 343-403 protein. The positive and negative control proteins, consisting of

15

the full length hypersensitive response elicitor protein and only background proteins, respectively, were tested for expression and HR activity as well.

The large scale expression and purification of the fragment proteins was done to determine the level of expression and titer of the HR activity (Table 3).

Table 3

Expression level and HR titer of hypersensitive response elicitor truncated proteins (large scale purification)

Fragment #	Amino acids (SEQ. ID. No. 23)	Expression	HR titer
1(+ control)	1-403	3.7 mg/ml	5-7 µg/ml
2 (- control)	-	-	1:2 dilution
4	169-403	2 mg/ml	-
5	210-403	5 mg/ml	-
6	267-403	4 mg/ml	-
7	343-402	200µg/ml	-
8	1-75	50µg/ml	-
9	1-104	50µg/ml	3 µg/ml (1:16 dilution)
10	1-168	1 mg/ml	1 µg/ml
13	76-209	2.5 mg/ml	5 µg/ml
14	76-168	2 mg/ml	-
15	105-209	5 mg/ml	5-10µg/ml
17	105-168	250µg/ml	-
19	137-204	3.6 mg/ml	3.5 µg/ml
20	137-180	250 µg/ml	16 µg/ml

The truncated proteins deemed to be the most important in characterizing the hypersensitive response elicitor were chosen for large scale expression. The positive control (full length hypersensitive response elicitor) was expressed at a relatively high level at 3.7 mg/ml. All of the C-terminal proteins were expressed at relatively high levels from 2-5 mg/ml, except for fragment 343-403 as discussed earlier. The N-terminal fragments were expressed very well also; however, during the purification process, the protein precipitated and very little was resolubilized. The concentrations in Table 3 reflect only the solubilized protein. The internal fragments were expressed in the range of 2-3.6 mg/ml. It was extremely difficult to determine the concentration of fragment 105-168 (it was suspected that the concentration was much higher than indicated), as the protein bands on the SDS gel were large, but poorly stained. The

- 53 -

negative control contained several background proteins as expected, but no obviously induced dominant protein.

### **Example 12 - Induction of HR in Tobacco**

5           The full length positive control protein elicited HR down to only 5-7 µg/ml. The negative control (pET 28) imidazole purified "protein" - which contained only background proteins - elicited an HR response down to the 1:2 dilution, which lowered the sensitivity of the assay as the 1:1 and 1:2 dilutions could  
10 not be used. This false HR was likely due to an affinity of the imidazole used in the purification process to bind to one or several of the background proteins, thereby not completely dialyzing out. Imidazole at a concentration of ca. 60 mM did elicit a false HR response.

          One definitive domain encompassing a small internal region of the  
15 protein from a.a. 137-180 (SEQ. ID. No. 23), a mere 44 a.a, is identified as the smallest HR domain. The other potential HR domain is thought to be located in the N-terminus of the protein from a.a. 1-104 (possibly a.a. 1-75) (SEQ. ID. No. 23). It was difficult to confirm or narrow down the N-terminus HR domain due to the difficulties encountered in purifying these fragment proteins. The N-terminus  
20 fragment proteins had to be purified with urea as no protein was recovered when the native purification process was used. Consequently, these proteins precipitated during the renaturing process and were difficult or nearly impossible to get back into solution, thereby making it hard to run the proteins through the HR assay, as only soluble protein is able to elicit HR. Difficulty narrowing the N-terminus HR domain  
25 was only compounded by the fact that the negative control elicited false HR at the low dilution levels thereby reducing the sensitivity of the assay.

          Surprisingly, when the internal HR domain was cleaved between a.a. 168 and 169 (fragments 76-168 and 105-168) (SEQ. ID. No. 23) the fragment lost its HR activity. This suggests that the HR activity of fragment 1-168 (SEQ. ID. No. 23)  
30 should not be attributed to the internal HR domain, but rather to some other domain, leading to the assumption that there was likely a second HR domain to be found in the N-terminal region of the protein. However, as discussed earlier it was difficult to confirm this assumption.

- 54 -

The hypersensitive response elicitor C-terminus (a.a. 210-403 (SEQ. ID. No. 23)) did not contain an HR domain. It did not elicit HR at a detectable level using the current HR assay. Even the large C-terminal fragment from a.a. 169-403 (SEQ. ID. No. 23) did not elicit HR even though it contained part of the internal HR domain. As stated above, cleaving the protein between amino acids 168 and 169 (SEQ. ID. No. 23) causes a loss of HR activity.

Because some of the small cloned proteins with 61 a.a. or less were not expressed, several oligopeptides were synthesized with 30 a.a. to narrow down the functional region of the internal HR domain. The oligopeptides were synthesized within the range of a.a. 121-179 (SEQ. ID. No. 23). However, these oligos did not elicit HR. It was not expected that there would be an HR from oligos 137-166, 121-150, and 137-156 (SEQ. ID. No. 23) as these fragments did not contain the imperative amino acids 168 and 169 (SEQ. ID. No. 23). It was expected that the oligo 150-179 (SEQ. ID. No. 23) would elicit an HR. It is possible that 30 a.a. is too small for the protein to elicit any activity due to a lack of folding and, therefore, a lack of binding or that during the synthesis of the peptides important amino acids were missed (either in the process, or simply by the choice of which 30 amino acids to synthesize) and, therefore, the fragments would not be able to elicit HR.

### 20 **Example 13 – Induction of Plant Growth Enhancement (PGE)**

The C-terminal fragments enhanced the growth of tomato by 9% to 21%. The N-terminal fragments enhanced the growth of tomato by 4% to 13%. The internal fragments enhanced growth by 9% to 20%. The 76-209 fragment enhanced growth by 18% at a concentration of 60 µg/ml, but not at the typical 20 µg/ml. This was attributed to the inaccuracy of the quantification process (Table 4).

- 55 -

Table 4

Fragment #	Amino acids	PGE ht>buffer @ 10 µg/ml	PGE ht>buffer @ 20 µg/ml
1 (+ control)	1-403	12%	11%
2 (- control)	-	-3%	-2%
4	169-403	9%	12%
5	210-403	13%	14% 16% @ 40µg/ml
6	267-403	21%	21% 23% @ 40µg/ml
7	343-403	7%	7%
9	1-104	4%	8%
10	1-168	13%	5%
13	76-209	7%	4% 18% @ 60µg/ml
14	76-168	18%	20%
15	105-209	14%	19%
17	105-168	19%	16%
19	137-204	11%	13%
20	137-180	--	9%

\*A height greater than 10% above the buffer control was necessary to pass the PGE assay.

The oligopeptides enhanced growth from 7.4% to 17.3% (Table 5).

Table 5

Fragment	Amino acids	Expression	HR titer	TMV efficacy	PGE ht>buffer
oligo	150-179	NA	-	72.9%	10.1%
oligo	137-166	NA	-	61.2%	12.0%
oligo	121-150	NA	-	60.0%	17.3%
oligo	137-156	NA	-	-87.7%	7.4%

The data suggests that there is more than one PGE domain, although the C-terminal and internal domains appear to be dominant over the N-terminal domain, as the N-terminal fragments enhanced growth the least amount.

#### **Example 14 – Induction of Systemic Acquired Resistance (SAR)**

All of the hypersensitive response elicitor fragments tested to date appear to have 60% efficacy or greater, except for the oligopeptide 137-156 (Tables 5 and 6).

- 56 -

Table 6

Fragment #	Amino acids	Efficacy of TMV control
1 (+ control)	1-403	84% & 72%
2 (- control)	-	40% & 31%
4	169-403	64% & 79%
5	210-403	77% and 78%
6	267-403	70% and 72%
9	1-104	82%
10	1-168	69%
13	76-209	44% and 84%
14	76-168	83% & 87%
15	105-209	57% and 67%
17	105-168	89%
19	137-204	89% & 77%
20	137-180	64% & 58%

These data suggest that there are multiple SAR domains within the protein.

#### **Example 15 – Relationship Between HR, PGE, and SAR**

It is clear that the hypersensitive response activity is separable from the plant growth enhancement activity. The C-terminal fragments clearly enhance the growth of tomato by ca. 20% at a concentration of only 20 µg/ml, but these same fragments were not able to elicit HR in tobacco, even at higher concentrations than 200 µg/ml. The SAR activity also appears to be separable from the HR activity. This finding is highly significant for future work on transgenic applications of the hypersensitive response elicitor technology. The fragments that induce PGE and/or SAR but do not elicit HR will be imperative for this technology, as constitutive expression of even low levels of an HR elicitor might kill a plant.

#### **Example 16 - Non-HR Eliciting Fragments Derived from the Hypersensitive Response Elicitor from *Pseudomonas syringae* pv. *syringae* Induce Resistance in Tobacco to TMV and Promote the Growth of Tomato**

To test whether non-HR eliciting fragments derived from HrpZ, the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, is able to induce disease resistance, several fragment constructs were made and the expressed

- 57 -

fragment proteins were tested for HR elicitation and disease resistance induction in tobacco and growth promotion in tomato.

The following segments of *hrpZ*, the gene encoding the hypersensitive response elicitor from *Pseudomonas syringae* pv. *syringae*, were amplified by PCR using Pfu Turbo (Stratagene): Regions coding for amino acids 152-190, aa 152-294, aa 190-294, aa 301-341, and full length HrpZ (aa 1-341). The DNA fragments were cloned into pCAL-n (Stratagene) to create C-terminal fusion proteins to the calmodulin-binding peptide. pCAL-n was chosen, because the fusion protein could be easily and gently purified on calmodulin resin. The DNA was transformed into *E. coli* DH5 $\alpha$ , and the correct clones were identified. The clones were then transferred to *E. coli* BLR DE3 for protein expression. The bacteria were grown in Terrific Broth to an OD<sub>620</sub> of 0.8-1.0. Protein expression was then induced with IPTG and the bacteria were incubated for an additional 3 h. All of the HrpZ fragments were able to be expressed this way.

Amino acid fragments 152-294 and 190-294 were chosen for further analysis and characterization. It was expected that the fragment 152-294 contained a domain that elicited the HR, while fragment 190-294 contained no domain that elicited the HR. The cultures were spun down, and the bacteria resuspended in 40 ml of 10 mM Tris pH 8.0. Twenty  $\mu$ l of antifoam and 40  $\mu$ l of 200 mM PMSF were added, and the bacteria was sonicated to break open the cells. The bacterial debris was removed by centrifugation, and the supernatant was placed in a boiling water bath for 10 min. The precipitate was removed by centrifugation and the supernatant, a crude protein preparation, was retained for tests.

Fifteen  $\mu$ l of each supernatant was run on a gel and stained to determine if the protein was present. It was estimated that about five times as much of the 152-294 fragment was present as the 190-294 fragment. Several dilutions of each preparation were infiltrated into tobacco leaves on two plants for HR tests (Table 7). As shown in Table 7, the 152-294 fragment elicited an HR, but the 190-294 fragment did not.

- 58 -

Table 7

HR test results of HrpZ fragments

<u>HrpZ Fragment</u>	<u>Dilution of Fragment Preparation<sup>a</sup></u>			
	<u>1:2</u>	<u>1:5</u>	<u>1:25</u>	<u>1:125</u>
152-294	+,+ <sup>b</sup>	+,+	+,+	-, -
190-294	-,-	-,-	-,-	-,-

<sup>a</sup> The preparations were diluted with MilliQ water.<sup>b</sup> The results are indicated for each of two plants. +, HR; -, no HR.

The fragment preparations were then tested for inducing resistance to TMV and for growth enhancement. Due to the difference in concentration of the HrpZ fragments, the 152-294 preparation was diluted 40-fold and the 190-294 preparation was diluted 8-fold. The results showed that the 190-294 aa fragment reduced the number of TMV lesions by 85% in comparison to buffer controls (Table 8). In contrast, the 152-294 aa fragment reduced the number of TMV lesions by only 55%. As also shown in Table 8, plants treated with the 152-294 aa fragment grew 4.64% more than buffer treated plants, while plants treated with the 190-294 aa fragment grew 2.62% more than the buffer treated plants.

Table 8

HR test, TMV, and PGE test results

<u>HrpZ Fragment</u>	<u>HR elicitation<sup>a</sup></u>	<u>TMV (% efficacy)<sup>b</sup></u>	<u>PGE(% &gt; buffer ht)<sup>c</sup></u>
152-294	+	54.64	4.64
190-294	-	85.25	2.62

<sup>a</sup> +, elicits HR in tobacco leaves; -, no HR in tobacco leaves.<sup>b</sup> % reduction in TMV lesions in unsprayed leaf of tobacco.<sup>c</sup> % greater height than buffer sprayed plants.

The results of these tests show that amino acids 152-190 appear to be involved in HR elicitation, because their removal eliminated the ability to elicit the HR. Both fragment preparations achieved disease control and growth enhancement. Thus, the ability to elicit the HR is not the determining factor for reduction in TMV infection and growth enhancement.



- 59 -

**Example 17 - Use of 13 Amino Acid Peptide Derived from *Phytophthora megasperma* Stimulates Tomato Seedling Growth**

Parsley leaves develop a typical resistance reaction against the soybean pathogen *Phytophthora megasperma* comprising hypersensitive cell death, defense related gene activation, and phytoalexin formulation. Several years ago, a 42 kDa glycoprotein elicitor was purified from the fungal culture filtrate of *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f.sp. glycinea Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991), which is hereby incorporated by reference). Then, an oligopeptide of 13 amino acid was identified within the 42-kDa glycoprotein. The 13-amino-acids-peptide appeared to have similar biological activity as that of the full length glycoprotein (42 kDa). It is sufficient to elicit a complex defense response in parsley cells including H<sup>+</sup>/Ca<sup>2+</sup> influxes, K<sup>+</sup>/Cl<sup>-</sup> effluxes, active oxygen production, SAR gene induction, and phytoalexin compound accumulation (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Response," Cell 78:449-460 (1994), which is hereby incorporated by reference).

To test if the 13 amino acid peptide derived from the 42 kDa protein also enhanced plant growth, 20 mg of the oligopeptide was synthesized from Biosynthesis Corp. The synthesized sequence of the peptide is NH<sub>2</sub>-Val-Trp-Asn-Gln-Pro-Val-Arg-Gly-Phe-Lys-Val-Tyr-Glu-COOH (SEQ. ID. No. 39). The synthesized peptide was resuspended in 10 ml of 5 mM potassium phosphate buffer and, then, diluted to 1 and 100 ng/ml with the same buffer. About 100 tomato seeds (variety, Marglobe) were submerged in 20 ml of peptide solution overnight. The soaked seeds were planted in an 8 inch pot with artificial soil. Seeds soaked in the buffer without the peptide were used as a control. After seedlings emerged and the first two true leaves fully expanded, the height of the tomato seedlings was recorded. The peptide was not able to elicit the HR in tobacco and other tested plants. However, it had a profound effect on plant growth promotion. Table 9 shows that tomato seedlings treated with the peptide increased 12.6 % in height, indicating that the fungal peptide derived from the 42 kDa glycoprotein can promote tomato seedling growth. Extended studies showed that the peptide also had similar growth

- 60 -

effect in other crops including tobacco. Similar growth promotion effects were achieved by plants sprayed with the peptide solution.

Table 9

5	Treatment	Height of seedlings (cm)					Average (cm) % Change	
	Buffer	6.0	6.0	6.0	5.5	5.5	5.55	-
		5.5	5.5	5.0	5.0	5.5		
10	Peptide Solution (100ng/ml)	6.5	6.0	6.5	6.5	6.5	6.25	12.6
		6.0	6.0	6.0	6.0	6.5		

15           Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

- 61 -

**WHAT IS CLAIMED:**

1. An isolated fragment of a hypersensitive response elicitor protein or polypeptide, wherein said fragment does not elicit a hypersensitive response but has other activity in plants.  
5
2. An isolated fragment according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas*, *Xanthomonas*, or *Phytophthora*.  
10
3. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.  
15
4. An isolated fragment according to claim 3, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.  
20
5. An isolated fragment according to claim 4, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.  
25
6. An isolated fragment according to claim 4, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.
7. An isolated fragment according to claim 4, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.  
30

- 62 -

8. An isolated fragment according to claim 2, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.
9. An isolated fragment according to claim 8, wherein the  
5 fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.
10. An isolated DNA molecule encoding a fragment according to claim 1.
- 10 11. An isolated DNA molecule according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from an *Erwinia Pseudomonas*, *Xanthomonas*, or *Phytophthora*.
- 15 12. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.
- 20 13. An isolated DNA molecule according to claim 12, wherein the fragment is selected from the group consisting of a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23, and an internal fragment of the amino acid sequence of SEQ. ID. No. 23.
- 25 14. An isolated DNA molecule according to claim 12, wherein the fragment is a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 169 and 403, 210 and 403, 267 and 403, or 343 and 403.
- 30 15. An isolated DNA molecule according to claim 12, wherein the fragment is an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 23.

- 63 -

16. An isolated DNA molecule according to claim 12, wherein the fragment is an internal fragment of the amino acid sequence of SEQ. ID. No. 23 spanning the following amino acids of SEQ. ID. No. 23: 105 and 179, 137 and 166, 121 and 150, or 137 and 156.

5

17. An isolated DNA molecule according to claim 11, wherein the hypersensitive response elicitor is derived from *Pseudomonas syringae*.

18. An isolated DNA molecule according to claim 18, wherein the  
10 fragment contains amino acids 190 to 294 of SEQ. ID. No. 31.

19. An expression system transformed with a DNA molecule according to claim 10.

20. An expression system according to claim 19, wherein said  
15 DNA molecule is in proper sense orientation and correct reading frame.

21. A host cell transformed with a DNA molecule according to  
claim 10.

20

22. A host cell according to claim 21, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

23. A host cell according to claim 21, wherein the DNA molecule  
25 is transformed with an expression system.

24. A transgenic plant transformed with the DNA molecule of  
claim 10.

25. A transgenic plant according to claim 24, wherein the plant is  
30 selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive,

- 64 -

cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5

26. A transgenic plant according to claim 24, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

10

27. A transgenic plant seed transformed with the DNA molecule of claim 10.

15

28. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

29. A transgenic plant seed according to claim 27, wherein the plant seed is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25

30. A method of imparting disease resistance to plants comprising: applying a fragment of a hypersensitive response elicitor protein or polypeptide, which fragment does not elicit a hypersensitive response, in a non-infectious form to a plant or plant seed under conditions effective to impart disease resistance.

30

31. A method according to claim 30, wherein plants are treated during said applying.

- 65 -

32. A method according to claim 30 wherein plant seeds are treated during said applying, said method further comprising:

5                   planting the seeds treated with the fragment of the  
hypersensitive response elicitor in natural or artificial soil and  
                  propagating plants from the seeds planted in the soil.

33. A method of enhancing plant growth comprising:  
                  applying a fragment of a hypersensitive response elicitor  
10   protein or polypeptide, which fragment does not elicit a hypersensitive response, in a  
non-infectious form to a plant or plant seed under conditions effective to enhance  
plant growth.

34. A method according to claim 33, wherein plants are treated  
15   during said applying.

35. A method according to claim 33, wherein plant seeds are  
treated during said applying, said method further comprising:  
                  planting the seeds treated with the fragment of the  
20   hypersensitive response elicitor in natural or artificial soil and  
                  propagating plants from the seeds planted in the soil.

36. A method of insect control for plants comprising:  
                  applying a fragment of a hypersensitive response elicitor protein or  
25   polypeptide, which fragment does not elicit a hypersensitive response, in a non-  
infectious form to a plant or plant seed under conditions effective to control insects.

37. A method according to claim 36, wherein plants are treated  
during said applying.

30

38. A method according to claim 36, wherein plant seeds are  
treated during said applying, said method further comprising:

- 66 -

planting the seeds treated with the fragment of the  
hypersensitive response elicitor in natural or artificial soil and  
propagating plants from the seeds planted in the soil.

5                   39.    A method of imparting disease resistance to plants comprising:  
                      providing a transgenic plant or plant seed transformed with a  
DNA molecule which encodes a fragment of a hypersensitive response elicitor protein  
or polypeptide, which fragment does not elicit a hypersensitive response, and  
                      growing the transgenic plant or transgenic plants produced  
10   from the transgenic plant seeds under conditions effective to impart disease resistance.

                  40.    A method according to claim 39, wherein a transgenic plant is  
provided.

15                  41.    A method according to claim 39, wherein a transgenic plant  
seed is provided.

                  42.    A method of enhancing plant growth comprising:  
                      providing a transgenic plant or a plant seed transformed with a  
20   DNA molecule which encodes a fragment of a hypersensitive response elicitor protein  
or polypeptide, which fragment does not elicit a hypersensitive response, and  
                      growing the transgenic plant or transgenic plants produced  
from the transgenic plant seeds under conditions effective to enhance plant growth.

25                  43.    A method according to claim 42, wherein a transgenic plant is  
provided.

                  44.    A method according to claim 42, wherein a transgenic plant  
seed is provided.

30                  45.    A method of insect control for plants comprising:



- 67 -

providing a transgenic plant or plant seed transformed with a  
DNA molecule which encodes a fragment of a hypersensitive response elicitor protein  
or polypeptide, which fragment does not elicit a hypersensitive response, and  
growing the transgenic plant or transgenic plants produced  
5 from the transgenic plant seeds under conditions effective to control insects.

46. A method according to claim 45, wherein a transgenic plant is  
provided.

10 47. A method according to claim 45, wherein a transgenic plant  
seed is provided.

---



1/2

#1	HARPIN			
	1			403
#3		C-TERMINAL FRAGMENTS		
#4		105		403
#5			169	403
#6			210	403
#7			267	403
			343	403
#8	N-TERMINAL FRAGMENTS			
#9	1	75		
#10	1	104		
#11	1		168	
#12	1		266	
	1			342
#13		INTERNAL FRAGMENTS		
#14		76		209
#15		76	168	
#16		105		209
#17			169	209
		105	168	
#18	SYNTHESIZED OLIGOPEPTIDES			
#19		99	209	
#20			137 204	150 179
#21			137 180	137 166
#22		105	180	121 150
#23			150 209	137 156
			150 180	

HARPIN FRAGMENTS DERIVED FROM HrpN OF ERWINIA AMYLOVORA

**FIG. 1****SUBSTITUTE SHEET (RULE 26)**



2/2

N1; 5'-GGGAATTCATATGAGTCTGAATACAAGTGGG-3'  
N76; 5'-GGGAATTCATATGGGCGGTGGCTTAGGCGGT-3'  
N99; 5'-GGCATATGTCTGAACGCGCTGAACGATATG-3'  
N105; 5'-GGGAATTCATATGTTAGGCGGTTCGCTGAAC-3'  
N110; 5'-GGCATATGCTGAACACGCTGGGCTCGAAA-3'  
N137; 5'-GGCATATGTCAACGTCCCAAACGACGAT-3'  
N150; 5'-GGCATATGTCCACCTCAGACTCCAGCG-3'  
N169; 5'-GGGAATTCATATGCAAAGCCTGTTTGGTGATGGG-3'  
N210; 5'-GGGAATTCATATGGGTAATGGTCTGAGCAAG-3'  
N267; 5'-GGGAATTCATATGAAAGCGGGCATTTCAGGCG-3'  
N343; 5'-GGGAATTCATATGACACCAGCCAGTATGGAGCAG-3'  
C75; 5'-GCAAGCTTAACAGCCCACCACCGCCCATCAT-3'  
C104; 5'-GCAAGCTTAAATCGTTCAGCGCGTTCGACAG-3'  
C168; 5'-GCAAGCTTAATATCTCGCTGAACATCTTCAGCAG-3'  
C180; 5'-GCAAGCTTAAGGTGCCATCTTGCCCATCAC-3'  
C204; 5'-GCAAGCTTAAATCAGTGACTCCTTTTTTATAGGC-3'  
C209; 5'-GCAAGCTTAACAGGCCCGACAGCGCATCAGT-3'  
C266; 5'-GCAAGCTTAAACCGATACCGGTACCCACGGC-3'  
C342; 5'-GCAAGCTTAATCCGTCGTCATCTGGCTTGCTCAG-3'  
C403; 5'-GCAAGCTTAAGCCGCGCCCAGCTTG-3'

---

OLIGONUCLEOTIDE PRIMERS USED FOR THE CONSTRUCTION  
OF THE SUBCLONES OF ERWINIA AMYLOVORA HrpN

**FIG. 2**



## SEQUENCE LISTING

<110> Eden Bioscience Corporation

<120> HYPERSENSITIVE RESPONSE ELICITOR FRAGMENTS WHICH ARE  
ACTIVE BUT DO NOT ELICIT A HYPERSENSITIVE RESPONSE

<130> 21829/32

<140>

<141>

<150> 60/103,050

<151> 1998-10-05

<160> 39

<170> PatentIn Ver. 2.0

<210> 1

<211> 31

<212> DNA

<213> Erwinia amylovora

<400> 1

gggaattcat atgagtctga atacaagtgg g

31

<210> 2

<211> 31

<212> DNA

<213> Erwinia amylovora

<400> 2

gggaattcat atgggcggtg gcttaggcgg t

31

<210> 3

<211> 29

<212> DNA

<213> Erwinia amylovora

<400> 3

ggcatatgtc gaacgcgctg aacgatatg

29

<210> 4

<211> 31

<212> DNA

<213> Erwinia amylovora





<400> 4  
gggaattcat atgttaggcg gttcgctgaa c

31

<210> 5  
<211> 29  
<212> DNA  
<213> Erwinia amylovora

<400> 5  
ggcatatgct gaacacgctg ggctcgaaa

29

<210> 6  
<211> 29  
<212> DNA  
<213> Erwinia amylovora

<400> 6  
ggcatatgtc aacgtcccaa aacgacgat

29

<210> 7  
<211> 27  
<212> DNA  
<213> Erwinia amylovora

<400> 7  
ggcatatgtc cacctcagac tccagcg

27

<210> 8  
<211> 34  
<212> DNA  
<213> Erwinia amylovora

<400> 8  
gggaattcat atgcaaagcc tgtttggtga tggg

34

<210> 9  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 9  
gggaattcat atgggtaatg gtctgagcaa g

31

<210> 10  
<211> 31  
<212> DNA  
<213> Erwinia amylovora



<400> 10  
gggaattcat atgaaagcgg gcattcaggc g 31

<210> 11  
<211> 34  
<212> DNA  
<213> Erwinia amylovora

<400> 11  
gggaattcat atgacaccag ccagtatgga gcag 34

<210> 12  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 12  
gcaagcttaa cagcccacca cgcgccatca t 31

<210> 13  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 13  
gcaagcttaa atcggtcagc gcgttcgaca g 31

<210> 14  
<211> 34  
<212> DNA  
<213> Erwinia amylovora

<400> 14  
gcaagcttaa tatctcgctg aacatcttca gcag 34

---

<210> 15  
<211> 30  
<212> DNA  
<213> Erwinia amylovora

<400> 15  
gcaagcttaa ggtgccatct tgcccatcac 30

<210> 16  
<211> 34  
<212> DNA  
<213> Erwinia amylovora



<400> 16  
gcaagcttaa atcagtgact ccttttttat aggc 34

<210> 17  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 17  
gcaagcttaa caggcccgac agcgcatcag t 31

<210> 18  
<211> 31  
<212> DNA  
<213> Erwinia amylovora

<400> 18  
gcaagcttaa accgataccg gtacccacgg c 31

<210> 19  
<211> 34  
<212> DNA  
<213> Erwinia amylovora

<400> 19  
gcaagcttaa tccgtcgtca tctggcttgc tcag 34

<210> 20  
<211> 25  
<212> DNA  
<213> Erwinia amylovora

<400> 20  
gcaagcttaa gccgcgccca gcttg 25

<210> 21  
<211> 338  
<212> PRT  
<213> Erwinia chrysanthemi

<400> 21  
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser  
1 5 10 15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser  
20 25 30

Leu Gly Ser S r Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr



35	40	45
Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu		
50	55	60
Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser		
65	70	75
Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys		
85	90	95
Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp		
100	105	110
Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln		
115	120	125
Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met		
130	135	140
Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly		
145	150	155
Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly		
165	170	175
Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu		
180	185	190
Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala		
195	200	205
Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val		
210	215	220

---

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp		
225	230	235
Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp		
245	250	255
Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys		
260	265	270
Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln		
275	280	285
Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr		





290

295

300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
 305 310 315 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
 325 330 335

Asn Ala

&lt;210&gt; 22

&lt;211&gt; 2141

&lt;212&gt; DNA

<213> *Erwinia chrysanthemi*

&lt;400&gt; 22

cgattttacc cgggtgaacg tgctatgacc gacagcatca cggatttcga caccgttacg 60  
 gcgttttatgg ccgcatgaa ccggcatcag gcggcgcgct ggtcgccgca atccggcgctc 120  
 gatctggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccgggg 180  
 cagcaatata ccggcatggt gcgcacgctg ctgcgtcgtc gttatcagca ggcggcagag 240  
 tgcgatggct gccatctgtg cctgaacggc agcgtatgtat tgatcctctg gtggcgcgctg 300  
 ccgtcggtac ccggcagtta tccgcagggt atcgaacggt tgtttgaact ggcgggaatg 360  
 acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc 420  
 cgatcattaa gataaaggcg gcttttttta ttgcaaaacg gtaacgggtg ggaaccggtt 480  
 caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgctg 540  
 ggcatccgtt gcagataact ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca 600  
 aattacgata aaagcgacac tcggcggtga tttgggcgtc tccggtctgg ggctgggtgc 660  
 tcagggactg aaaggactga attccgcggc ttcacgctg ggttccagcg tggataaact 720  
 gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct 780  
 ggcgcgaggg ctgggcgcca gctcgaaggg gctggggatg agcaatcaac tgggccagtc 840  
 tttcggaat ggcgcgagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcgggcg 900  
 tgcgttgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960  
 caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020  
 ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgctgt ccattctcgg 1080  
 caacgggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggtt 1140  
 gcagggcctg agcggcgcgg gtgcattcaa ccagttgggt aatgccatcg gcatgggcgt 1200  
 ggggcagaat gctgcgctga gtgcgttgag taacgtcagc acccagtag acggtaacaa 1260  
 ccgccacttt gtagataaag aagatcgcg catggcgaaa gagatcgcc agtttatgga 1320  
 tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380  
 gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg 1440  
 cgccagcatg gacaaattcc gtcaggcgat gggatatgat aaaagcgcg tggcggtgga 1500  
 taccggcaat accaacctga acctgcgtgg cgcgggcggt gcatcgctgg gtatcgatgc 1560  
 ggctgtcgtc ggcgataaaa tagccaacat gtcgctgggt aagctggcca acgcctgata 1620  
 atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680  
 ttattatgag gtttatggg ttacctggac cggttaatca tcgtcatcga tctggtacaa 1740  
 acgcacattt tcccgttcat tcgcgtcggt acgcgccaca atcgcgatgg catcttctc 1800



gtcgcgcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc 1860  
 cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccttttag 1920  
 cagatagatt gcggtttcgt aatcaacatg gtaatgcggg tccgcctgtg cgccggccgg 1980  
 gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgccgg agataccgac 2040  
 aaaatagggc agtttttgcg tggatatccgt ggggtgttcc ggcctgacaa tcttgagttg 2100  
 gtctgcatc atctttctcc atctgggcga cctgacggg t 2141

<210> 23

<211> 403

<212> PRT

<213> *Erwinia amylovora*

<400> 23

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser  
 1 5 10 15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln  
 20 25 30

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn  
 35 40 45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met  
 50 55 60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu  
 65 70 75 80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu  
 85 90 95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr  
 100 105 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro  
 115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser  
 130 135 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln  
 145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly  
 165 170 175

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu  
 180 185 190



Gly Glu Gln Asn Ala Tyr Lys Lys ly Val Thr Asp Ala Leu Ser Gly  
 195 200 205  
 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly  
 210 215 220  
 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu  
 225 230 235 240  
 Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln  
 245 250 255  
 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln  
 260 265 270  
 Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe  
 275 280 285  
 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met  
 290 295 300  
 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro  
 305 310 315 320  
 Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser  
 325 330 335  
 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365

---

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
 370 375 380  
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
 385 390 395 400  
 Gly Ala Ala

<210> 24  
 <211> 1288  
 <212> DNA  
 <213> Erwinia amylov ra



&lt;400&gt; 24

```

aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa 60
gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct 120
atcggcgggtg cgggcggaataaacgggttg ctgggtacca gtcgccagaa tgctgggttg 180
ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg 240
gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcgggtg tgggctgatg 300
ggcgggtggc taggcgggtg cttaggtaat ggcttgggtg gctcagggtg cctgggcgaa 360
ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa 420
ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct gggattatac 480
tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac 540
ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tggatgatggg 600
caagatggca cccaggcgag ttccctctggg ggcaagcagc cgaccgaagg cgagcagaac 660
gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag 720
ctccttggca acgggggact gggaggtggg cagggcggta atgctggcac gggctctgac 780
ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccgggtgga ctaccagcag 840
ttaggtaacg ccgtgggtac cgggtatcggg atgaaagcgg gcattcaggc gctgaatgat 900
atcgggtacg acaggcacag ttcaacctcg tcttctgtca ataaaggcga tcgggcgatg 960
gcgaaggaaa tcgggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac 1020
cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080
aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140
atgatcaaaa ggcccatggc ggggtgatacc ggcaacggca acctgcaggc acgcgggtgcc 1200
gggtggttctt cgctgggtat tgatgccatg atggccgggtg atgccattaa caatatggca 1260
cttggaagc tgggcgcggc ttaagctt

```

&lt;210&gt; 25

&lt;211&gt; 1344

&lt;212&gt; DNA

<213> *Erwinia amylovora*

&lt;400&gt; 25

```

atgtcaattc ttacgttaa caacaatacc tegtctcgc cgggtctgtt ccagtcggg 60
ggggacaacg ggcttgggtg tcataatgca aattctgcgt tggggcaaca accatcgat 120
cggcaaacca ttgagcaaat ggtcaatta ttggcggaac tgtaaagtc actgctatcg 180
ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttgg 240
aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa cactccgca gtctgacagt 300
cagaacatgc tgagtgagat gggcaacaac gggctggatc aggccatcac gcccgatggc 360
cagggcggcg ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca 420
cgcagatggg acggccaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc 480
tcttccggta cttcttcacg tggcggttcc ccttttaacg atctatcagg ggggaaggcc 540
ccttccggca actcccttc cggcaactac tctcccgta gtacctctc accccatcc 600
acgccaacgt cccctacctc accgcttgat tcccttctt ctcccacaa agcagccggg 660
ggcagcacgc cggtaaccga tcatectgac cctgttggta gcgcgggcat cggggccgga 720
aatcgggtgg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc 780
gtgaaagcgg gtcaggtgtt tgatggcaaa ggacaaacct tcaccgcccg ttcagaatta 840
ggcgatggcg gccagttctg aaaccagaaa ccgctgttta tactggaaga cgggtgccagc 900
ctgaaaaacg tcaccatggg cgacgacggg gcggtgggta ttcattctta cgggtgatgcc 960
aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020

```





agcgcgggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac 1080  
 aagatcctgc agctgaatgc cgatactaac ctgagcgttg acaacgtgaa ggccaaagac 1140  
 tttggtactt ttgtacgcac taacggcggt caacagggtg actgggatct gaatctgagc 1200  
 catatcagcg cagaagacgg taagttctcg ttcgttaaaa gcgatagcga ggggctaaac 1260  
 gtcaatacca gtgatctctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320  
 gccaacctga aggtggctga atga 1344

<210> 26

<211> 447

<212> PRT

<213> *Erwinia amylovora*

<400> 26

Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu  
 1 5 10 15

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser  
 20 25 30

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala  
 35 40 45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly  
 50 55 60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly  
 65 70 75 80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro  
 85 90 95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu  
 100 105 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp  
 115 120 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp  
 130 135 140

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala  
 145 150 155 160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
 165 170 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
 180 185 190



Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pr  
 195 200 205  
 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
 210 215 220  
 Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly  
 225 230 235 240  
 Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His  
 245 250 255  
 Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln  
 260 265 270  
 Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn  
 275 280 285  
 Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val  
 290 295 300  
 Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala  
 305 310 315 320  
 Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr  
 325 330 335  
 Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn  
 340 345 350  
 Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp  
 355 360 365  


---

 Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe  
 370 375 380  
 Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser  
 385 390 395 400  
 His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser  
 405 410 415  
 Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu  
 420 425 430  
 Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu  
 435 440 445



&lt;210&gt; 27

&lt;211&gt; 5517

&lt;212&gt; DNA

<213> *Erwinia amylovora*

&lt;400&gt; 27

```

atggaattaa aatcactggg aactgaacac aaggcggcag tacacacagc ggcgcacaac 60
cctgtggggc atggtgttgc cttacagcag ggcagcagca gcagcagccc gcaaaatgcc 120
gctgcatcat tggcggcaga aggcaaaaat cgtgggaaaa tgccgagaat tcaccagcca 180
tctactgcgg ctgatggtat cagcgtctgt caccagcaaa agaaatcctt cagtctcagg 240
ggctgttttg ggacgaaaaa attttccaga tcggcaccgc agggccagcc aggtaccacc 300
cacagcaaaag gggcaacatt gcgcgatctg ctggcgcggg acgacggcga aacgcagcat 360
gaggcggccg cgccagatgc ggcggtttg acccgttcgg gcggcgtaa acgccgcaat 420
atggacgaca tggccggggcg gccaatggtg aaaggtggca gcggcgaaag taaggtacca 480
acgcagcaaa aacggcatca gctgaacaat tttggccaga tgcggcaaac gatgttgagc 540
aaaatggctc acccggttcc agccaacgcc ggcgatcgcc tgacgattc accgccgcac 600
atcccggtta gccaccacga aatcaaggaa gaaccggttg gctccaccag caaggcaaca 660
acggcccacg cagacagagt ggaaatcgct caggaagatg acgacagcga attccagcaa 720
ctgcatcaac agcggctggc gcgcgaacgg gaaaatccac cgcagccgcc caaactcggc 780
gttgccacac cgattagcgc caggtttcag cccaaactga ctgcggttgc ggaaagcgtc 840
cttgagggga cagataccac gcagtcaccc cttaagccgc aatcaatgct gaaaggaagt 900
ggagccgggg taacgcgct ggcggtaacg ctggataaag gcaagttgca gctggcaccg 960
gataatccac ccgcgtcaa tacgttgtt aaagcagacat tgggtaaaga caccagcac 1020
tatctggcgc accatgccag cagcgacggt agccagcatc tgctgctgga caacaaaggc 1080
cacctgtttg atatcaaaag caccgccacc agctatagcg tgctgcacaa cagccacccc 1140
ggtgagataa agggcaagct ggcgaggcg ggtactggct ccgtcagcgt agacggtaaa 1200
agcggcaaga tctcgctggg gagcggtacg caaagtcaca acaaaacaat gctaagccaa 1260
ccgggggaag cgcaccgttc cttattaacc ggcatttggc agcatcctgc tggcgagcgc 1320
cggcgcaggg gcgagtcatt ccgcctgcat gacgacaaaa ttcatactct gcacccggag 1380
ctgggcgtat ggcaatctgc ggataaagat acccacagcc agctgtctcg ccaggcagac 1440
ggtaagctct atgcgctgaa agacaaccgt accctgcaaa acctctccga taataaatcc 1500
tcagaaaagc tggctcgataa aatcaaatcg tattccgttg atcagcgggg gcagggtggc 1560
atcctgacgg atactcccgg ccgccataag atgagtatta tgccctcgct ggatgcttcc 1620
ccggagagcc atatttccct cagcctgcat tttgccgatg cccaccaggg gttattgcac 1680
gggaagtcgg agcttgaggc acaatctgtc gcgatcagcc atgggcgact ggttgtggcc 1740
gatagcgaag gcaagctggt tagcgccgcc attccgaagc aaggggatgg aaacgaactg 1800
aaaatgaaag ccatgcctca gcctgcgctc gatgaacatt ttggtcatga ccaccagatt 1860
tctggatttt tccatgacga ccacggccag cttaatgcgc tgggtgaaaa taacttcagg 1920
cagcagcatg cctgcccgtt gggtaacgat catcagtttc accccggctg gaacctgact 1980
gatgcgctgg ttatcgacaa tcagctgggg ctgcatcata ccaatcctga accgcagtag 2040
attcttgata tggggcattt aggcagcctg gcgttacagg agggcaagct tcactatttt 2100
gaccagctga ccaaaggggt gactggcgcg gactcagatt gtaagcagct gaaaaaaggc 2160
ctggatggag cagcttatct actgaaagac ggtgaagtga aacgcctgaa tattaatcag 2220
agcacctcct ctatcaagca cggaacggaa aacgtttttt cgctgccgca tgtgcgcaat 2280
aaaccggagc cgggagatgc cctgcaaggg ctgaataaag acgataaggc ccaggccatg 2340
gcgggtgattt gggtaataaa atacctggcg ctgacggaaa aaggggacat tcgctccttc 2400

```



cagataaaac ccggcaccaca gcagttggag cggccggcac aaactctcag ccgcgaagggt 2460  
 atcagcggcg aactgaaaga cattcatgtc gaccacaagc agaacctgta tgccttgacc 2520  
 cacgagggag aggtgtttca tcagccgcgt gaagcctggc agaatgggtc cgaaagcagc 2580  
 agctggcaca aactggcggt gccacagagt gaaagtaagc taaaaagtct ggacatgagc 2640  
 catgagcaca aaccgattgc cacctttgaa gacggtagcc agcatcagct gaaggctggc 2700  
 ggctggcacg cctatgcggc acctgaacgc gggccgctgg cgggtgggtac cagcggttca 2760  
 caaacctgtc ttaaccgact aatgcagggg gtgaaaggca aggtgatccc aggcagcggg 2820  
 ttgacggtta agctctcggc tcagacgggg ggaatgaccg gcgcgaagg gcgcaaggtc 2880  
 agcagtaaat tttccgaaag gatccgcgcc tatgcgttca acccaacaat gtccacgccg 2940  
 cgaccgatta aaaatgctgc ttatgccaca cagcacggct ggagggggcg tgaggggttg 3000  
 aagccgttgt acgagatgca gggagcgctg attaaacaac tggatgcgca taacgttcgt 3060  
 cataacgcgc cacagccaga tttgcagagc aaactggaaa ctctggattt aggcgaacat 3120  
 ggcgcagaat tgcttaacga catgaagcgc ttccgcgacg aactggagca gagtgcaccc 3180  
 cgttcgggtga ccgttttagg tcaacatcag ggagtgttaa aaagcaacgg tgaaatcaat 3240  
 agcgaattta agccatcgcc cggcaaggcg ttggtccaga gctttaacgt caatcgctct 3300  
 ggtcaggatc taagcaagtc actgcaacag gcagtacatg ccacgccgcc atccgcagag 3360  
 agtaaacctgc aatccatgct ggggcacttt gtcagtgcgc ggggtggatat gaggcatcag 3420  
 aaggcgagga tcccgctggg ccgccagcgc gatccgaatg ataaaaccgc actgacaaa 3480  
 tcgcgtttta ttttagatac cgtgaccatc ggtgaactgc atgaactggc cgataaggcg 3540  
 aaactgggtat ctgaccataa acccgatgcc gatcagataa aacagctgcg ccagcagttc 3600  
 gatacgctgc gtgaaaagcg gtatgagagc aatccgggtg agcattacac cgatatgggc 3660  
 ttcaccata ataaggcgct ggaagcaaac tatgatgcgg tcaaagcctt tatcaatgcc 3720  
 tttagaaaag agcaccacgg cgtcaatctg accacgcgta ccgtactgga atcacaggcg 3780  
 agtgcgagc tggcgaagaa gctcaagaat acgctgttgt ccctggacag tggtgaaagt 3840  
 atgagcttca gccggtcata tggcgggggc gtcagcactg tctttgtgcc tacccttagc 3900  
 aagaagggtc cagttccggt gatccccgga gccggcatca cgctggatcg cgcctataac 3960  
 ctgagcttca gtcgtaccag cggcggattg aacgtcagtt ttggccgcga cggcgggggtg 4020  
 agtggttaaca tcatggtcgc taccggccat gatgtgatgc cctatatgac cggtaagaaa 4080  
 accagtgcag gtaacgccag tgactgggtg agcgcaaac ataaaatcag cccggacttg 4140  
 cgtatcgcg cgtgctgtgag tggcaccctg caaggaacgc taaaaaacag cctgaagttt 4200  
 aagctgacag aggatgagct gcctggcttt atccatggct tgacgcattg cacgttgacc 4260  
 ccggcagaa tggttgcaaaa ggggatcgaa catcagatga agcagggcag caaactgacg 4320  
 tttagcgtcg atacctcggc aaatctggat ctgcgtgccg gtatcaatct gaacgaagac 4380  
 ggcagtaaac caaatgggtg cactgcccggt gtttctgccg ggctaagtgc atcggaac 4440  
 ctggccgcgc gctcgcgtga acgcagcacc acctctggcc agtttggcag cacgacttcg 4500  
 gccagcaata accgccaac ctctctcaac ggggtcggcg cgggtgctaa cctgacggct 4560  
 gctttagggg ttgccattc atctacgcat gaagggaac cggtcgggat cttcccgga 4620  
 tttacctga ccaatgtttc ggcagcgtg gcgctggata accgtacctc acagagtatc 4680  
 agcctggaat tgaagcgcgc ggagcgggtg accagcaacg atatcagcga gttgacctcc 4740  
 acgctgggaa aacactttaa ggatagcgcc acaacgaaga tgcttgccgc tctcaaagag 4800  
 ttagatgacg ctaagccgcg tgaacaactg catattttac agcagcattt cagtgcacaa 4860  
 gatgtcgtcg gtgatgaacg ctacgaggcg gtgcgcaacc tgaaaaaact ggtgatacgt 4920  
 caacaggctg cggacagcca cagcatggaa ttaggatctg ccagtcacag cacgacctac 4980  
 aataatctgt cgagaataaa taatgacggc attgtcgagc tgctacacaa acatttcgat 5040  
 gcggcattac cagcaagcag tgccaaacgt cttgggtgaa tgatgaataa cgatccggca 5100  
 ctgaaagata ttattaagca gctgcaaagt acgccgttca gcagcgccag cgtgtcgatg 5160  
 gagtgaaag atggtctgcg tgagcagacg gaaaaagcaa tactggacgg taaggctcgt 5220  
 cgtgaagaag tgggagtact tttccaggat cgtaacaact tgcgtgttaa atcggtcagc 5280





gtcagtcagt ccgtcagcaa aagcgaaggc ttcaataccc cagcgctggt actggggacg 5340  
 agcaacagcg ctgctatgag catggagcgc aacatcggaa ccattaattt taaatacggc 5400  
 caggatcaga acaccccacg gcgatttacc ctggagggtg gaatagctca ggctaataccg 5460  
 caggtcgcat ctgcgcttac tgatttgaag aaggaagggc tggaaatgaa gagctaa 5517

<210> 28

<211> 1838

<212> PRT

<213> *Erwinia amylovora*

<400> 28

Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr  
 1 5 10 15

Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser  
 20 25 30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly  
 35 40 45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala  
 50 55 60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg  
 65 70 75 80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln  
 85 90 95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala  
 100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala  
 115 120 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met  
 130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro  
 145 150 155 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln  
 165 170 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp  
 180 185 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile



195	200	205
Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala		
210	215	220
Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln		
225	230	235 240
Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro		
	245	250 255
Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys		
	260	265 270
Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln		
	275	280 285
Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val		
	290	295 300
Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro		
	305	310 315 320
Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys		
	325	330 335
Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln		
	340	345 350
His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr		
	355	360 365
Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys		
	370	375 380
Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys		
	385	390 395 400
Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr		
	405	410 415
Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile		
	420	425 430
Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg		
	435	440 445
Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp		



450		455		460
Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp				
465		470		480
Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser				
	485		490	495
Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser				
	500		505	510
Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg				
	515		520	525
His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His				
	530		535	540
Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His				
	545		550	555
Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg				
	565		570	575
Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro				
	580		585	590
Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His				
	595		600	605
Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe				
	610		615	620
His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg				
	625		630	635
Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly				
	645		650	655
Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His				
	660		665	670
His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly				
	675		680	685
Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr				
	690		695	700
Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly				



705	710	715	720
Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu	725	730	735
Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val	740	745	750
Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu	755	760	765
Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly	770	775	780
Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe	785	790	795
Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu	805	810	815
Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His	820	825	830
Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln	835	840	845
Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys	850	855	860
Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser	865	870	875
His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln	885	890	895

---

Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro	900	905	910
Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met	915	920	925
Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys	930	935	940
Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val	945	950	955
Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr			





965	970	975
Met Ser Thr Pro Arg Pr	Ile Lys Asn Ala Ala Tyr Ala Thr Gln His	
980	985	990
Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly		
995	1000	1005
Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro		
1010	1015	1020
Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His		
1025	1030	1035
Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu		
1045	1050	1055
Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val		
1060	1065	1070
Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly		
1075	1080	1085
Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu		
1090	1095	1100
Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu		
1105	1110	1115
Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp		
1125	1130	1135
Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro		
1140	1145	1150
Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val		
1155	1160	1165
Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser		
1170	1175	1180
Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe		
1185	1190	1195
Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr		
1205	1210	1215
Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp		



1220	1225	1230
Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val		
1235	1240	1245
Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu		
1250	1255	1260
Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser		
1265	1270	1275 1280
Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val		
1285	1290	1295
Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly		
1300	1305	1310
Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly		
1315	1320	1325
Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile		
1330	1335	1340
Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys		
1345	1350	1355 1360
Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile		
1365	1370	1375
Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly		
1380	1385	1390
Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro		
1395	1400	1405

---

Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu		
1410	1415	1420
Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr		
1425	1430	1435 1440
Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn		
1445	1450	1455
Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser		
1460	1465	1470
Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg		



1475	1480	1485
Ser Thr Thr S r Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn		
1490	1495	1500
Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala		
1505	1510	1515 1520
Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly		
1525	1530	1535
Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu		
1540	1545	1550
Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu		
1555	1560	1565
Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys		
1570	1575	1580
His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu		
1585	1590	1595 1600
Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His		
1605	1610	1615
Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg		
1620	1625	1630
Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser		
1635	1640	1645
Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser		
1650	1655	1660
Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp		
1665	1670	1675 1680
Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn		
1685	1690	1695
Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro		
1700	1705	1710
Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu		
1715	1720	1725
Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val		



1730                      1735                      1740

Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser  
 1745                      1750                      1755                      1760

Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu  
                     1765                      1770                      1775

Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile  
                     1780                      1785                      1790

Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg  
                     1795                      1800                      1805

Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser  
                     1810                      1815                      1820

Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser  
                     1825                      1830                      1835

&lt;210&gt; 29

&lt;211&gt; 420

&lt;212&gt; DNA

<213> *Erwinia amylovora*

&lt;400&gt; 29

atgacatcgt cacagcagcg ggttgaaagg tttttacagt atttctccgc cgggtgtaaa 60  
 acgcccatac atctgaaaga cggggtgtgc gccctgtata acgaacaaga tgaggaggcg 120  
 gcggtgctgg aagtaccgca acacagcgac agcctgttac tacactgccg aatcattgag 180  
 gctgaccac aaacttcaat aaccctgtat tcgatgctat tacagctgaa ttttgaaatg 240  
 gcggccatgc gcggtgttg gctggcgctg gatgaactgc acaacgtgcg tttatgtttt 300  
 cagcagtcgc tggagcatct ggatgaagca agtttttagcg atatcgtag cggcttcac 360  
 gaacatgcgg cagaagtgcg tgagtatata gcgcaattag acgagagtag cgcggcataa 420

&lt;210&gt; 30

&lt;211&gt; 139

&lt;212&gt; PRT

<213> *Erwinia amylovora*

&lt;400&gt; 30

Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser  
                     1                      5                      10                      15

Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu  
                     20                      25                      30

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His





35                                      40                                      45  
 Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pr Gln  
 50                                      55                                      60  
 Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met  
 65                                      70                                      75                                      80  
 Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val  
 85                                      90                                      95  
 Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe  
 100                                      105                                      110  
 Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu  
 115                                      120                                      125  
 Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala  
 130                                      135

<210> 31  
 <211> 341  
 <212> PRT  
 <213> *Pseudomonas syringae*

<400> 31  
 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1                                      5                                      10                                      15  
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
 20                                      25                                      30  
 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
 35                                      40                                      45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
 50                                      55                                      60  
 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
 65                                      70                                      75                                      80  
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85                                      90                                      95  
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100                                      105                                      110



Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
 180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
 195 200 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
 210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
 225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
 245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
 260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
 275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325 330 335

Asn Gln Ala Ala Ala  
 340

<210> 32

<211> 1026



&lt;212&gt; DNA

<213> *Pseudomonas syringae*

&lt;400&gt; 32

```

atgcagagtc tcagtcttaa cagcagctcg ctgcaaaccg cggcaatggc ccttgtcctg 60
gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgttca ggaagtgtgc 120
gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga 180
aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcggtat tgaggatgtc 240
atcgctgcgc tggacaagct gatccatgaa aagctcgggtg acaacttcgg cgcgtctgcg 300
gacagcgccg cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc 360
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac 420
gatatgccga tgctgaacaa gatcgcgcag ttcattggatg acaatcccgc acagtttccc 480
aagccggact cgggtcctcg ggtgaacgaa ctcaaggaa acaacttcct tgatggcgac 540
gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag 600
agtgcgctg gcagtcctggc agggacgggt ggaggtctgg gcactccgag cagtttttcc 660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggctc cggtgacagc 720
ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgcagac cggtagctcg 840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggtct gctgctcaag 900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960
gcgcaaactc ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca 1020
gcctga 1026

```

&lt;210&gt; 33

&lt;211&gt; 1729

&lt;212&gt; DNA

<213> *Pseudomonas syringae*

&lt;400&gt; 33

```

tccacttcgc tgattttgaa attggcagat tcatagaaac gttcaggtgt ggaaatcagg 60
ctgagtgcgc agatttcggt gataaggggt tggtagtggc cattgttggc catttcaagg 120
cctctgagtg cgggtgcggag caataccagt ctctctgctg gcgtgtgcac actgagtcgc 180
aggcataggg atttcagttc cttgcgttgg ttgggcatat aaaaaaagga acttttaaaa 240
acagtgcaat gagatgccgg caaaacggga accggtcgct gcgctttgcc actcacttcg 300
agcaagctca accccaaaca tccacatccc tatcgaacgg acagcgatac ggccacttgc 360
tctggtaaac cctggagctg gcgtcgggtc aattgcccac ttagcgaggt aacgcagcat 420
gagcatcggc atcacacccc ggccgcaaca gaccaccagc cactcgtatt ttccggcgct 480
aagcggcaag agtccctaac caaacacggt cggcgagcag aacactcagc aagcgatcga 540
cccagagtgc ctgttggttc gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600
cagcaccgtc cagaatccgc aggcagccag caagcccaac gacagccagt ccaacatcgc 660
taaattgata agtgacattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa 720
gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcggggt 780
cggtagacccg tcggccgata gcggggggcg cggtagacccg gatgcgacag gtggcgggcg 840
cggtgatacg ccaagcgcaa caggcgggtg cggcggtgat actccgaccg caacaggcgg 900
tggcggcagc ggtggcgggc gcacacccac tgcaacaggt ggcggcagcg gtggcacacc 960
cactgcaaca ggcggtggcg aggggtggcg aacaccgcaa atcactccgc agttggccaa 1020
ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080
cggcaagatc aatgtggtga aagacacat caaggtcggc gctggcgaag tctttgacgg 1140

```



ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200  
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260  
cgaggctgat ggcattccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320  
gcatgccag aacgtcgggtg aagacctgat tacggtcaaa ggcgagggag gcgcagcgggt 1380  
cactaatctg aacatcaaga acagcagtgac caaagggtgca gacgacaagg ttgtccagct 1440  
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatgggt 1500  
tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcacgaagc 1560  
taaccacggc aagttcgccc tggtgaaaag cgacagtgac gatctgaagc tggcaacggg 1620  
caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgaccaaca 1680  
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc 1729

&lt;210&gt; 34

&lt;211&gt; 424

&lt;212&gt; PRT

<213> *Pseudomonas syringae*

&lt;400&gt; 34

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu

1 5 10 15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly

20 25 30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly

35 40 45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val

50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile

65 70 75 80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr

85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln

100 105 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser

115 120 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Asp Thr

130 135 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly

145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly





165	170	175
Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr		
180	185	190
Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr		
195	200	205
Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile		
210	215	220
Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp		
225	230	235
Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp		
245	250	255
Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr		
260	265	270
Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val		
275	280	285
Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln		
290	295	300
Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala		
305	310	315
Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp		
325	330	335
Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe		
340	345	350

---

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln		
355	360	365
Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly		
370	375	380
Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr		
385	390	395
Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln		
405	410	415
Ala Ser Thr Gln His Thr Glu Leu		



420

&lt;210&gt; 35

&lt;211&gt; 344

&lt;212&gt; PRT

&lt;213&gt; Pseudomonas solanacearum

&lt;220&gt;

<223> Description of Unknown Organism: Pseudomonas  
solanacearum

&lt;400&gt; 35

Met	Ser	Val	Gly	Asn	Ile	Gln	Ser	Pro	Ser	Asn	Leu	Pro	Gly	Leu	Gln
1				5					10					15	

Asn	Leu	Asn	Leu	Asn	Thr	Asn	Thr	Asn	Ser	Gln	Gln	Ser	Gly	Gln	Ser
			20					25					30		

Val	Gln	Asp	Leu	Ile	Lys	Gln	Val	Glu	Lys	Asp	Ile	Leu	Asn	Ile	Ile
		35					40					45			

Ala	Ala	Leu	Val	Gln	Lys	Ala	Ala	Gln	Ser	Ala	Gly	Gly	Asn	Thr	Gly
	50					55					60				

Asn	Thr	Gly	Asn	Ala	Pro	Ala	Lys	Asp	Gly	Asn	Ala	Asn	Ala	Gly	Ala
65					70					75					80

Asn	Asp	Pro	Ser	Lys	Asn	Asp	Pro	Ser	Lys	Ser	Gln	Ala	Pro	Gln	Ser
				85					90					95	

Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met
			100					105					110		

Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala
		115					120					125			

Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val
	130					135					140				

Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala
145					150					155				160	

Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly
			165					170					175		

Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly
			180				185						190		



Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala  
195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn  
210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn  
245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln  
260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly  
275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser  
290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln  
325 330 335

Gln Ser Thr Ser Thr Gln Pro Met  
340

<210> 36

<211> 1035

<212> DNA

<213> *Pseudomonas solanacearum*

<400> 36

atgtcagtcg gaaacatcca gagcccgctcg aacctcccgg gtctgcagaa cctgaacctc 60  
aacaccaaca ccaacagcca gcaatcgggc cagtcctgtgc aagacctgat caagcaggtc 120  
gagaaggaca tcctcaacat catcgagcc ctcgtgcaga aggccgcaca gtcggcgggc 180  
ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgcaa cgcggggcgcc 240  
aacgaccgga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc 300  
ggcaacgtcg acgacgcaa caaccaggat cccgatgcaag cgctgatgca gctgctggaa 360  
gacctggtga agctgctgaa ggcgccctcg cacatgcagc agcccgggcg caatgacaag 420  
ggcaacggcg tgggcggtgc caacggcgcc aagggtgccc gcggccaggc cggcctggcc 480  
gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcgggcg tgctggcgcc 540  
ggcgggcgcg gtggcggtgt cggcggtgt ggtggcgcg atggcgggctc cgggtcggggt 600



```

ggcgcaggcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac 660
ggcccgcaga acgcaggcga tgtcaacggg gccaacggcg cggatgacgg cagcgaagac 720
cagggcgggc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctgggtgcag 780
atgatgcagc aaggcggcct cggcggcggc aaccaggcgc agggcggctc gaagggtgcc 840
ggcaacgcct cgccggcttc cggcgcgaac ccgggcgcga accagcccgg ttcggcggat 900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960
gtccagatcc tgcagcagat gctggcggcg cagaacggcg gcagccagca gtccacctcg 1020
acgcagccga tgtaa 1035

```

&lt;210&gt; 37

&lt;211&gt; 26

&lt;212&gt; PRT

<213> *Xanthomonas campestris* pv. *glycines*

&lt;400&gt; 37

```

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
  1             5             10             15

```

```

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
          20             25

```

&lt;210&gt; 38

&lt;211&gt; 20

&lt;212&gt; PRT

<213> *Xanthomonas campestris* pv. *pelargonii*

&lt;400&gt; 38

```

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
  1             5             10             15

```

```

Leu Leu Ala Met
          20

```

&lt;210&gt; 39

&lt;211&gt; 13

&lt;212&gt; PRT

<213> *Phytophthora megasperma*

&lt;400&gt; 39

```

Val Trp Asn Gln Pro Val Arg Gly Phe Lys Val Tyr Glu
  1             5             10

```





# INTERNATIONAL SEARCH REPORT

Int. Application No  
PCT/US 99/23181

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/195 C12N15/31 C12N1/21 C12N5/10 A01H5/00  
A01H5/10 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NÜRNBERGER T, ET AL. : "High Affinity Binding of a Fungal Oligopeptide Elicitor to arslay Plasma Membranes Triggers Multiple Defense Responses" CELL, vol. 78, no. 3, 12 August 1994 (1994-08-12), pages 449-460, XP000882736 Cambridge, Mass. cited in the application the whole document	1,2,10, 11, 19-23, 30-32, 36-38
A	WO 98 32844 A (CORNELL RES FOUNDATION INC) 30 July 1998 (1998-07-30) the whole document -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

6 March 2000

Date of mailing of the international search report

03/04/2000

Name and mailing address of the ISA

European Patent Office, P.B. 6818 Patentaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Bilang, J

Int. Application No.  
PCT/US 99/23181

<sup>1</sup> Application No

**PCT/us 99/23181**

[illegible]

# INTERNATIONAL SEARCH REPORT

in connection with patent family members

Int. Application No.  
PCT/US 99/23181

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
W0 9832844	A	30-07-1998	AU	6043198 A	18-08-1998
W0 9824297	A	11-06-1998	AU	5693598 A	29-06-1998
			EP	0957672 A	24-11-1999

